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The cover illustration, created by Professor Antony O'Hara of the Digital Multimedia Design Program, represents the gradual diminishing of mental function in Alzheimer's Disease. It reflects the theme of the first three articles.

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Molecular Mechanisms of Alzheimer's Disease

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Abstract

Alzheimer's disease was first discovered in 1906 by Alois Alzheimer. It is a neurodegenerative disease characterized by the buildup of toxic amyloid plaque and intracellular neurofibrillary tangles, which results in the progressive loss of cognitive function and memory. Since its discovery, the disease has become a growing health concern, particularly in the developed world, where the ageing demographics have contributed to an increase in its prevalence and incidence. The earliest research into the disease focused on neurochemical analyses and resulted in the formulation of the cholinergic hypothesis. The mechanism of disease was explained as the degeneration of the cholinergic system and a reduction in acetylcholine. While much data supports this hypothesis, it fails to explain the accumulation of amyloid plaque, a hallmark of the disease. Analysis of the genetic factors in familial Alzheimer's disease, and the discovery of the higher risk for Alzheimer's disease amongst individuals with Down's syndrome led to the more comprehensive amyloid cascade hypothesis. The failure of both amyloid centric drugs and cholinesterase inhibitors to have a significant impact on disease progression has caused some to have rejected both these hypotheses to focus on other possible causes. However, there is undoubtedly a wealth of data in support of both the cholinergic hypothesis and the amyloid cascade hypothesis. Understanding the functional relationship between the cholinergic system and the formation of beta amyloid plaques may lead to a greater understanding of the mechanism of disease and provide a target for more effective therapy.

Introduction

Alzheimer's disease was first identified by Alois Alzheimer in 1906. However not until the 1970's did it become a major and significant area of research. Since that time much has been discovered about the mechanisms of the disease, however the precise biological processes in the disease are mostly unknown and the large variance in its progression amongst patients with the disease needs to be better understood.

Alzheimer's disease is a neurodegenerative disease that effects memory, behavior and eventually leads to death within an average of 8 years of diagnosis, the last three of which are typically spent with full time care or in an institution.

The changing demographics worldwide and with the baby boom generation reaching ages 70 and beyond has led Alzheimer's disease to become one of the biggest healthcare concerns in the developed world. This greater prevalence and incidence of Alzheimer's disease, the largest cause of dementia, has created a huge burden on society.

Millions of Americans have Alzheimer's disease and other forms of dementias. An estimated 5.4 million Americans of all ages had Alzheimer's disease in 2016. One in nine people aged 65 years or older and about one third (32%) of people aged 85 and older have the disease (Herbert et al., 2013).

The future projections for Alzheimer's disease are equally bleak. Approximately 476,000 people will develop the disease in the United States in 2016, with the numbers increasing dramatically with age. There will be 63,000 new cases among people aged 65 to 74 and 241,000 new cases among people aged 85 and older. Because of the ageing demographics in the United States, these numbers are projected to double by 2050 (Herbert et al., 2001).

Alzheimer's disease is one of the leading causes of both mortality and morbidity in the United States. It is currently the sixth leading cause of death for those 65 years and older. According to data from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), 84,767 people died from it in 2013. These numbers only consider those who have cause of death listed as Alzheimer's disease on their death certificate. Death certificates for individuals with Alzheimer's disease often list other acute conditions, such as pneumonia, as the primary cause of death and therefore death due to Alzheimer's is most likely underreported.

The cost of the disease is very substantial, both in terms of the value of the unpaid caregiving for Alzheimer's disease which has been estimated at $221.3 billion in 2013, and the total payments made which in 2016 was estimated at $236 billion. Overall the cost of healthcare, long term care and hospice care for individuals with the disease makes it one of the costliest chronic diseases to society (Hurd et al., 2013).

Alzheimer's disease is characterized by a gradually worsening ability to remember new information. This occurs because the first neurons to be damaged and destroyed are in the area of the brain responsible for creating new memories. As the disease causes the destruction of neurons in other regions of the brain, symptoms worsen and individuals experience other difficulties such as challenges in problem solving and planning, confusion with time and place, decreased and poor judgement, and withdrawal from social activities and work.

There are about 100 billion neurons in the adult healthy brain. During the development of Alzheimer's disease, the connections and synapses between neurons is hindered and the overall number of neurons decrease. The destruction of neurons and the disruption of the cellular neuronal circuits lead to many of

the symptoms. The brains of people with advanced Alzheimer's disease show inflammation, dramatic shrinkage from cell loss and widespread debris from dead and destroyed neurons. Some of these brain changes can begin 20 years prior to the onset of symptoms for the disease (Villemagne et al., 2013).

No simple test currently exists for diagnosis of Alzheimer's disease, rather an individual's physician together with the help of a neurologist will use a variety of methods to assist in a diagnosis. These include obtaining a family history and medical history of the patient which may include psychiatric, cognitive and behavioral histories. In addition, diagnosis is often aided by conducting cognitive tests and physical and neurological examinations. Finally, a physician will typically use blood tests and brain imaging to rule out other possible causes of behavioral and memory changes such as tumor formation or nutrient deficiencies.

Much research has focused on a potential precursor to Alzheimer's disease known as Mild Cognitive Impairment (MCI). An individual with Mild Cognitive Impairment will experience mild but measurable changes in thinking abilities that are noticeable to friends and families of the affected person but that do not affect the person's ability to carry out normal everyday functions and activities (Roberts & Knopman, 2013). It is estimated that about 20% of people over the age of 65 have Mild Cognitive Impairment. Recent studies have shown that an average of 32% of people with MCI will develop Alzheimer's disease within 5 years. However, some with MCI will see their cognitive decline stabilize, and in some cases, they may even return to normal cognition (Ward et al., 2013).

In a small percent of those diagnosed with Alzheimer's disease the development of the disease can be attributed to genetic mutations. Three genes have been implicated in its development. These are the genes which encode for the amyloid precursor protein (APP), the genes for presenilin-1 and for presenilin-2. Mutations in both the APP gene and the presenilin-1 gene result in guaranteed development of the disease, while a mutation in the presenilin-2 gene leads to a 95% chance of its development. Individuals with mutations in any of these three genes will usually develop symptoms as young as age 30, unlike the vast majority of Alzheimer's cases, which are late onset where symptoms typically develop at age 65 and over (Bekris et al., 2010).

People with Down syndrome are born with an additional copy of chromosome 21 and have a greater risk of developing all forms of dementia including Alzheimer's disease. Studies have found that more than 75% of people with Down Syndrome aged 65 and over have Alzheimer's. While the exact relationship between Down Syndrome and Alzheimer's is not entirely clear, one possible explanation might be that the gene that codes for amyloid precursor protein is located on chromosome 21 and the additional copy of this gene increases the likelihood of the development of the plaques associated with the amyloid precursor protein. By age 40 most people with Down Syndrome have high levels of beta amyloid plaques in their brains, a marker for Alzheimer's disease (Lott & Dierssen, 2010).

Besides for the genetic factors in Alzheimer's disease much research has been conducted on the effects of environmental and modifiable risk factors. Studies have shown that regular physical activity, and management of cardiovascular risk factors such as obesity, smoking and high blood pressure reduce the risk of the development of Alzheimer's disease and dementia. There is also evidence that a healthy lifestyle and diet as well as continued engagement in learning can prevent cognitive decline in old age (Baumgart et al., 2015).

Current treatment options for Alzheimer's disease are limited. None of the pharmacological treatments available cures or stops the damage the disease causes to the brain. The six drugs that have thus far been approved by the U.S Food and Drug Administration (FDA) focus primarily on increasing the levels of neurotransmitter present in the brain. While this helps deal with the symptoms of Alzheimer's disease such as memory loss and reduced cognitive capacity, these drugs don't deal with the underlying issues in the disease, and their effectiveness is limited to the early stages of the disease.

This paper attempts to review some of the complex molecular mechanisms involved in Alzheimer's disease. It will explore the current research on the molecular mechanisms in Alzheimer's disease to better understand the development of the disease and the wide variance in disease progression, and explain possible areas of future research in the development of more effective therapeutic agents.

Methods

This study was performed through the analysis of various original and peer reviewed articles which were accessed using databases such as the Touro Database, PubMed, and Google Scholar. The research collected in this study was used to understand the molecular processes in Alzheimer's disease, and to evaluate the various hypotheses postulated in the formation of the disease.

Discussion

Since the systematic biochemical analysis of patients with Alzheimer's began in the early 1970's much knowledge has been acquired concerning the possible mechanisms responsible for the disease. The earliest research into neurochemical abnormalities that are present in Alzheimer's disease led to the formulation of the cholinergic hypothesis.

Cholinergic Hypothesis

Acetylcholine was the first neurotransmitter to be identified and is the neurotransmitter used in all cholinergic neurons. It is fundamental in both the central nervous system (CNS) and the peripheral nervous system (PNS).

Successful neurotransmission of acetylcholine is dependent on proteins needed for its synthesis, transport, degradation and reuptake. Acetylcholine synthesis takes place in the cytoplasm of cholinergic cells. The enzyme, choline acetyltransferase (ChAT) catalyzes the combination reaction of dietary choline and Acetyl-CoA, generated by the mitochondria, to form the product acetylcholine. Three forms of the ChAT enzyme have been found in humans. Formation of acetylcholine is followed by its transfer to the synaptic vesicles before release. This step is enabled by the vesicular acetylcholine transporter (VAChT). When the cholinergic neurons are depolarized, acetylcholine is released by exocytosis into the synaptic cleft. On the post synaptic neuron, acetylcholine can activate both muscarinic and nicotinic receptors. Nicotinic ACh receptors are ion gated channels which are selective for cations including sodium, potassium, and calcium. Nicotinic receptors are made up from a combination of five different subunits. The large variance in the properties and functions of the different nicotinic receptors is a direct result of the many different possible combinations of subunits that form each receptor. In the PNS the activation of these receptors results in the transmission of the signal to ganglion cells and the innervation of muscles and glands. In the CNS the role of nicotinic receptors is regulatory rather than purely the transmission of excitatory or inhibitory signals. In the PNS the nicotinic receptors are mostly located on the post synaptic neuronal membrane, where they are activated by acetylcholine and facilitate the transmission of signals. However, in the CNS the receptors are mostly located on the pre-synaptic neuronal membrane where their activation regulates the release of acetylcholine and other neurotransmitters into the synapse. The activation of these receptors results in an increase in calcium levels in the presynaptic neuron, which is a crucial step in the exocytosis of neurotransmitters such as GABA, glutamate, dopamine and serotonin into the synapse. Muscarinic receptors are G-protein coupled receptors, and unlike the nicotinic receptors, their role is largely in the transmission of signals. Five isoforms of the receptor have so far been identified. M1, M3 and M5 are excitatory receptors and their activation results in the formation of second messengers by phospholipase C which results in closure of K+ channels enabling the greater depolarization of the cell and the transmission of signal down the axon. M2 and M4 are inhibitory and their activation has the opposite effect. They result in the inhibition of adenylyl cyclase which leads to lower levels of cyclic adenosine monophosphate (cAMP) and promotes the inhibition of [Ca]++ channels diminishing cell excitability.

In the synaptic cleft acetylcholine is broken down to its components Acetyl-CoA and choline by the enzyme acetylcholinesterase (AChE). AChE is one of the most kinetically effective enzymes. It is able to catalyze the breakdown of 5000 molecules of acetylcholine per second. The choline transporter, CHT1, facilitates the uptake of choline, that is produced by the breakdown of acetylcholine by AChE, into the presynaptic neuron. This transporter is also the source of the choline used in acetylcholine synthesis and it therefore plays a crucial role in the recycling of acetylcholine (Fig. 1, Ferreira Vieira et al., 2016).

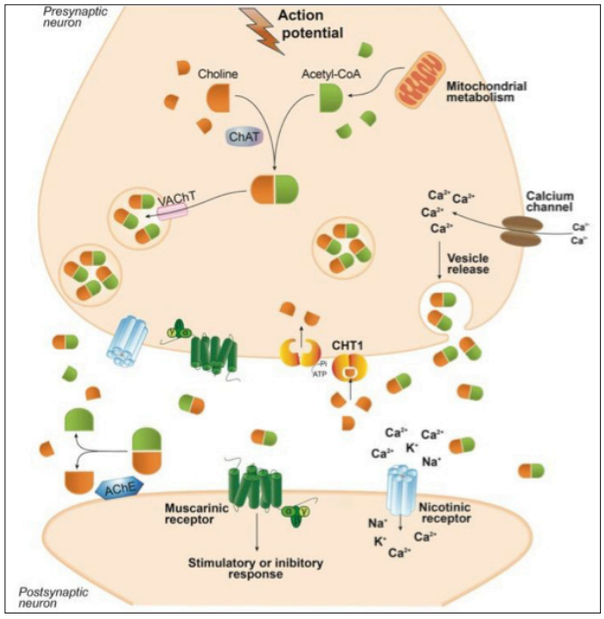

Figure 1 Schematic representation of biological aspects involving acetylcholine neurotransmission. (Ferreira Vieira et al., 2016)

Early research into Alzheimer's disease showed reduced activity of choline acetyltransferase in the amygdala, hippocampus and cortex of patients with the disease. In one such study, the levels of the enzyme responsible for acetylcholine synthesis in patients with the disease was found to be lower than 10% of the normal activity seen in the control group. Similarly, the levels of acetylcholinesterase in the same brain regions were markedly lower in patients with Alzheimer's disease (Davies & Maloney 1976).

Further studies showed that in addition to the decreased activity of the enzymes responsible for acetylcholine synthesis, the levels of acetylcholine uptake by neurons in the frontal cortex and in the hippocampus, were significantly lower in patients with Alzheimer's disease. In the frontal cortex, the transport of choline into synaptosomes was reduced by 50% and an even greater reduction of 80% was seen in the hippocampus. Further evidence of the loss of cholinergic function in the uptake of

choline in Alzheimer's disease is seen in the lower densities of the presynaptic high affinity choline uptake carrier (HACU) in both the cortex and hippocampus (Rylett et al., 1983; Pascual et al., 1991).

Other studies have shown there to be a reduction in the number of nicotinic and muscarinic M2 acetylcholine receptors in Alzheimer's disease brains and there is evidence for the disruption of the M1 receptors and their G-proteins which effects the second messenger systems and transmission of signals (Whitehouse et al., 1988).

The cholinergic hypothesis of Alzheimer's disease was also evidenced by the reduction in the amount of acetylcholine released from neurons in the brains of affected patients. An experiment conducted on brain tissue of both controls and patients with the disease collected with short post mortem delay, showed a significant reduction in tritium (3H-acetylcholine) release during potassium stimulation, as compared with the controls (Nilsson et al., 1986).

In addition, the loss of cholinergic neurons as a result of the neurodegeneration caused by Alzheimer's has further implicated the cholinergic system in the etiology of the disease. In particular, the selective degeneration of neurons in the nucleus basalis of Meynert, a significant source of neurons which are fundamental in the cholinergic innervation of the cerebral cortex, explains the reduction in the levels of acetylcholine in individuals with Alzheimer's disease (Whitehouse et al., 1982).

It has also been demonstrated that the cholinergic system plays a role in memory and in learning. The loss of the neurons in the nucleus basalis of Meynert has been correlated with the impaired memory and cognitive abilities seen in patients with Alzheimer's disease. The effects on the cholinergic system has also been shown to result in the many behavioral and psychological symptoms seen in individuals with the disease. Emotional processing deficits associated with Alzheimer's disease may be caused by loss of cholinergic function in the areas of the amygdala and frontal cortex. Apathy and depression as well as disturbance in sleep cycle are symptoms commonly found in patients with Alzheimer's disease. It has been postulated that the degeneration of cholinergic neurons and the resulting loss of regulation of neurotransmitters, including dopamine and serotonin, is responsible for the many psychiatric symptoms observed. Moreover, cholinesterase inhibitors, the primary focus of the current pharmacologic agents available for the treatment of Alzheimer's disease have shown to improve many of the psychiatric symptoms. Clinical trials conducted on affected patients, reported reduced delusions, stress, apathy and depression in the treated group (Ferreira et al., 2016; Pinto et al., 2011).

There is much research that shows the link between the cholinergic system and Alzheimer's disease. The neurodegeneration of neuronal cells critical in the cholinergic system, the reduction in the enzymes responsible for the synthesis of acetylcholine, and lower levels of proteins that facilitate choline uptake in neuronal cells that is seen in the brains of patients with Alzheimer's disease, together with the role that the cholinergic system plays in memory and learning have contributed to the formulation of the cholinergic hypothesis. However there remains some inconsistencies in this hypothesis that suggest the role of the cholinergic system in the disease needs to be better understood.

Although cholinergic loss seems to correlate with cognitive impairment, other factors such as loss of synapses and pyramidal cells may also be responsible for the cognitive decline. Additionally, some patients with Alzheimer's disease do not show the large decreases in ChAT activity that would be expected. Moreover, patients with inherited olivopontocerebellar activity have levels of ChAT which are reduced to similar levels as those seen in Alzheimer's patients yet they don't experience the decline in cognition and memory that would be expected.

An expectation of the cholinergic hypothesis would be that drugs that restore cholinergic function to the brain regions effected by Alzheimer's disease would improve and reverse the cognitive symptoms seen in the disease. Five drugs have been approved by the FDA for Alzheimer's disease. Four of these drugs are cholinesterase inhibitors and one is a receptor antagonist. The cholinesterase inhibitors have shown limited success in clinical trials and are mostly able to merely delay the progression of the disease. The failure of the cholinesterase inhibitors to cure Alzheimer's disease was seen by some as the strongest evidence against the cholinergic hypothesis.

Many researchers have continued to explore the role of neurotransmission in the disease because of the acetylcholine dysfunction seen in the brains of Alzheimer's disease patients. However, the inconsistencies with the cholinergic hypothesis have resulted in a shift in the focus of much of the more recent research towards two of the hallmarks of the disease, the buildup of beta amyloid plaques in the extracellular space, and the intracellular formation of neurofibrillary tangles (Craig et al., 2011; Francis et al., 1999).

Amyloid Cascade Hypothsis

The origins of the Amyloid Cascade Hypothesis lie in the sequencing of the Aβ extracted from cerebral blood vessels and the brain parenchyma of Alzheimer's disease patients. The identification of the Aβ sequence led to the sequencing of the amyloid precursor protein gene (APP). This gene located on chromosome 21 encodes the holoprotein that is cleaved first by

the β-amyloid cleaving enzyme and then by γ-secretase to form the Aβ peptide. (Masters et al., 1985; Kang et al., 1987; Hussain et al., 1999). The essence of the amyloid cascade hypothesis is that the increased production or decreased clearance of the Aβ peptides causes the disease. Aggregation of the hydrophobic Aβ40 and Aβ42 peptides results in the formation of insoluble plaque which triggers a cascade of changes ultimately resulting in cell death and the symptoms of the disease.

Human APP belongs to a family of type I transmembrane glycoproteins that also includes the similar amyloid precursor like proteins 1 and 2 (APLP1 and APLP2). These proteins are functionally the same as the APP but they lack the Aβ sequence. The APP gene is highly conserved and several alternatively spliced isoforms of APP have been identified in humans. Invertebrates such as the fruit fly D. melanogaster and the worm C. elegans contain paralogs of the amyloid precursor protein, amyloid protein precursor-like (APPL) and APP-like 1, (APL-1). The zebrafish genome encodes two variants of APP, Appa and Appb. All of these proteins share domains that are highly conserved in both the large extracellular domains and in the shorter cytoplasmic domain (Nicolas & Hassan, 2014).

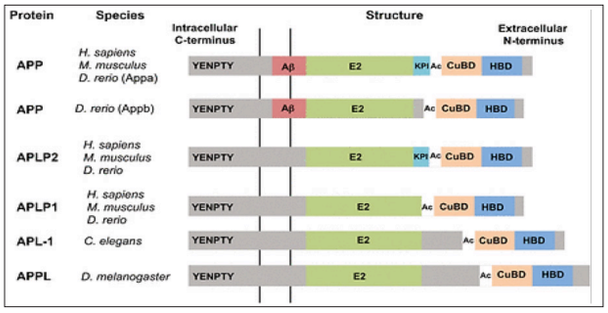

Figure 2 The domain structure of APP family members in model organisms. All APP homologs contain the extracellular domains E2, an acidic domain (Ac), a copper binding domain (CuBD), and a heparin binding domain (HBD). A kunitz protease inhibitor domain (KPI) is found only in the APP and APLP-2 forms of the protein and the Aβ sequence is only present in APP. The most highly conserved domain across species is the intracellular YENPTY domain (Nicolas & Hassan, 2014).

APP can undergo two types of processing depending on the secretases that cleave it. In the non-amyloidogenic pathway APP is cleaved within the Aβ sequence by α-secretase forming the sAPPα extracellular protein and the membrane bound αAPP-CT. The membrane bound protein is then cleaved by γ-secretase forming P3 peptide and amyloid precursor protein intracellular domain (AICD).

In the amyloidogenic pathway, APP is first cleaved by β-secretase forming the soluble sAPPβ protein and the C-terminal membrane bound fragment βAPP-CTF. The subsequent cleavage of the βAPP-CTF fragment by γ-secretase forms the Aβ peptide and the amyloid precursor intracellular domain (ACID). The release of the Aβ peptide by γ-secretase is thought to be fundamental to Alzheimer's disease pathology, and the aggregation of these insoluble and toxic protein fragments results in the formation of the senile plaque that is a hallmark of the disease. In the earliest and most direct elucidation of the amyloid cascade

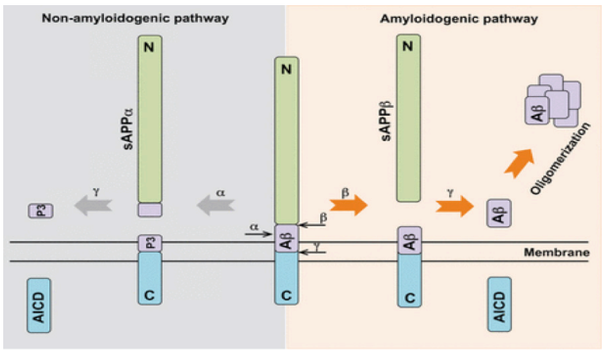

Figure 3 proteolytic processing of APP (Nicolas & Hassan, 2014).

hypothesis the aggregation of the Aβ peptide was explained as critical event in the development of Alzheimer's disease. The accumulation of the peptide was seen as both a cause of cell death and as a crucial step in the hyper-phosphorylation of Tau proteins leading to the formation of intracellular neurofibrillary tangles (Hardy & Higgins, 1992).

Critical evidence for the ACH comes from human genetics. The observation that the Aβ peptide deposited in elderly Down's syndrome patients, was the same as that found in patients with Alzheimer's suggested that a gene on chromosome 21 was central to the development of the disease (Glenner & Wong, 1984a). Later studies on a family with a history of early onset Alzheimer's provided further evidence for a genetic link in the disease, and was the basis for much of the research that followed. These studies revealed a missense mutation in the APP gene that resulted in a V717I amino acid substitution in the protein product. The position of the substitution, just upstream from the carboxyl terminal cleavage site of the Aβ peptide, provided further evidence for the role of Aβ peptide in the etiology of the disease (Goate et al., 1991).

The link between mutations in the presenilin 1 and presenilin 2 genes, that encode a part of the γ-secretase mutiprotein complex, and the development of the disease has likewise provided support for the amyloid cascade hypothesis.

There are now hundreds of mutations to the PSEN-1, PSEN-2 and APP genes that are known to cause early onset familial AD (FAD). These mutations effect the formation and accumulation of amyloid plaque in several ways. Some of these mutations result in

the extension of the C-terminal side of the Aβ peptide, others increase the overall ratio of the longer less soluble Aβ peptides to the shorter more soluble forms, and some are directly responsible for an increase in the aggregatory properties of the protein.

In addition to the effects of these three genes on familial Alzheimer's disease, there are also genes that are connected to sporadic Alzheimer's disease (SAD). There are three major alleles of the APOE gene in the human population (Nickerson et al., 2000). These are APOE2, APOE3 and APOE4. A heterozygous APOE4 carrier has a four-fold increased risk for the disease as compared with the homozygous APOE3 genotype. The homozygous APOE4 genotype has an even more drastic 12 fold increase in risk. Conversely, a carrier of the APOE2 gene has a reduced risk of the disease (Verghase et al., 2011). The ApoE protein is believed to have a role in the deposition of the amyloid plaques. Furthermore, there are specific mutations in the APP gene that result in the protection against Alzheimer's disease, with the evidence suggesting that they disrupt the ability to form the Aβ peptides.

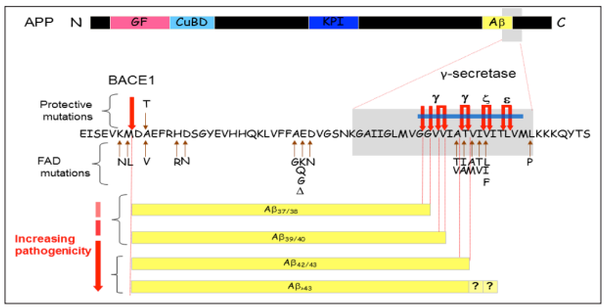

Figure 4 Amyloid precursor proteins. The diagram shows both the β- secretase and γ-secretase cleavage sites and protective and pathogenic mutations (Karran & De Strooper, 2016).

While many of the above studies have shown a link between the genes involved in the formation of the Aβ peptides and the development of the disease, like the cholinergic hypothesis there remain some inconsistencies and unanswered questions. These questions have led many to believe that rather than being the central mechanism in the development of Alzheimer's disease, the formation of amyloid plaque may just be one of several factors that contribute to its development.

Much to the frustration of the proponents of the ACH, the amyloidocentric drugs have failed to produce the results that would have been expected according to the hypothesis. The phase three clinical trials of 6 of these drugs have provided disappointing results. Triampirosate, a small molecule drug that prevents the aggregation of Aβ peptide showed no significant effect on cognition and memory during the clinical trials. Similarly no significant effect on primary outcomes were seen in the trials of Tarenflurbil, a γ-secretase modulator that reduces the ratio of aggregatory Aβ peptide to the shorter more soluble form. The only limited success in this class of drug for the treatment of Alzheimer's disease, was Solanezumab, a monoclonal antibody directed at the amyloid plaque protein. In its phase three clinical trials there was a significant improvement in cognitive abilities. In an extension trial, these positive effects on cognition were sustained over a two-year period raising hope and providing some evidence for a disease modifying effect (Karran & De Strooper, 2016).

A central tenet of the amyloid cascade hypothesis is that the accumulation of the Aβ peptides causes both cell death and the intracellular neurofibrillary tangles. Tissue studies in vitro have shown the toxic nature of Aβ peptide and Aβ42. Many early studies showed that the addition of the peptide to cell culture resulted in neuronal death and apoptosis as well as synaptic and dendritic loss. In vivo studies produced similar results, when these deleterious effects were seen in mouse brains injected with the peptide. However, the relationship between the formation of amyloid plaque and the tangles remains unclear. The aggregation of plaque initially takes place in the frontal cortex, before spreading throughout the cortex as the disease progresses. Neurofibrillary tangles first appear in the limbic system, in the hippocampus and dentate gyrus. This spatial discrepancy together with further studies that have shown that the tangles appear before the accumulation of plaque, and are more closely correlated with disease progression and severity have contributed to the re-evaluation of the ACH (Braak & Braak, 1998).

An analysis conducted to determine the correlation between cognitive abilities and some of the neurochemical and structural measurements in 15 patients with Alzheimer's disease further questions the relationship between amyloid plaque and cognitive impairment. In this study, there were only weak correlations between plaque and tangles and performance on psychometric tests. In contrast, the density of the cortical synapses was found to be strongly correlated with the psychological assessments of the patients (Terry et al., 1991). Mouse models of Alzheimer's disease have shown the accumulation of plaques without the observed cognitive impairments. In addition, neuroimaging analysis have shown the presence of plaques in cognitively healthy people.

The amyloid cascade hypothesis dominates the field of Alzheimer's research as the most complete and evidenced hypothesis about the causes of the disease. However, it is not without its flaws, which has led to some reassessing the hypothesis and to others dismissing its significance altogether. The genetic link to Alzheimer's disease was considered the central evidence for this hypothesis. Mutations in APP, PSEN1 and

PSEN2 are associated with familial Alzheimer's disease and the EPOE4 allele is a risk factor for sporadic AD. The fact that these genes are involved in the processing of APP and the formation of Aβ peptide has provided strong evidence for the role of amyloid plaque in disease progression. The mutations associated with APP are all located in the proximity of the cleavage sites of Aβ peptide, suggesting that the plaque formed by the peptide is central to the development of the disease. However, the mutations in the presenilin genes, which encode γ-secretase, occur throughout the protein, and not just the sites that are involved with Aβ formation. This has led to the belief that defective APP processing may in fact be the actual cause of the disease, and that the formation of the Aβ peptide and plaques might just be a secondary effect. This belief is supported by the weak correlation between plaque formation and density and cognition. While the ACH is the best defined and most widely accepted view, the data both for and against this hypothesis is significant. Many still strongly support the ACH and others have dismissed it entirely to focus on other hypotheses.

Alternative Hypotheses

Analysis of the relationship between mitochondrial function and Alzheimer's disease has resulted in the proposition of the mitochondrial cascade hypothesis (MCH). The fundamental principle of the MCH is that Alzheimer's disease is a usual if not inevitable consequence of ageing. Support for this hypothesis comes from the undoubted evidence of mitochondrial damage in the brains of patients with the disease. Mitochondria are critical in the regulation of cell death and mutations in mitochondrial DNA and oxidative stress both contribute to ageing and neurodegenerative diseases (Lin & Beal, 2006). Studies using flourodeoxyglucose positron emission tomography (PET) imaging, as a measure of oxygen uptake, showed deficits in the brains of Alzheimer's disease patients (Jack et al., 2012). There is also strong evidence of free radical damage in AD brains (Sonnen et al., 2008). These findings suggest that dysfunctional and altered mitochondria is central in the disease. Experimental evidence for the MCH comes from studies conducted on cybrid cells- cells that contains mitochondria from a different cell. Studies on cybrid cells which were induced to take up mitochondrial DNA from platelets of patients with Alzheimer's disease, showed increased production of beta amyloid peptide (Khan et al., 2000). This data provides support for the MCH, by showing that mitochondrial deficiency is the cause of the amyloid plaques and central to disease development. Despite this data, the MCH fails to explain the full array of Alzheimer's disease pathology. In addition, genome wide association studies have failed to show links between mitochondrial genes and proteins and the disease. The cybrid experiments show that mitochondrial dysregulation in Alzheimer's disease leads to increases in beta amyloid production. However, they do not account for the mutations in APP and PSEN which increase the ratio of insoluble Aβ peptide to the smaller soluble form, or the mutations that result in an increase in aggregatory properties.

Another hypothesis that deserves mention is the metabolism hypothesis. According to this hypothesis, the underlying cause of AD is hypometabolism of glucose in the brain. The basis of this hypothesis was experiments on a rat model injected with streptozotocin, which effects insulin production, and resulted in decreased glucose metabolism in the brain, and learning and memory impairments (Lannert & Hoyer 1998). Further studies showed that the insulin signaling pathway is significantly depressed in many brain regions in Alzheimer's disease (Steen et al., 2005). Clinical experiments also have linked insulin with Aβ peptide buildup. Incretin mimetics injected in mice resulted in significant reductions in Aβ plaque load (Mcclean & Hölscher, 2014), and there is also data that suggests that Aβ oligomers disrupt the insulin pathway leading to an increase in glycogen synthase kinase 3β (GSK 3β), a Tau kinase, and the formation of neurofibrillary tangles (Morgen & Frölich, 2015). As with the mitochondrial hypothesis, no data from genome wide association studies have confirmed this link between Alzheimer's disease and insulin signaling. However, this hypothesis does provide several targets for possible therapeutic intervention.

A more generalized proposition is the cell cycle re-entry hypothesis. This view examines Alzheimer's disease from the perspective of age related DNA damage. Neurons are post mitotic and therefore must maintain integrity long term. Brain cells have a very high energy requirement and therefore are particularly susceptible to DNA damage. Mitogen kinases have increased expression in the brains of Alzheimer's disease patients, and have been posited to stimulate the neurodegenerative pathways and effect cell repair mechanisms (Arendt et al., 1995). Later studies on differentiated neurons that were infected with the oncogenes, c-myc and ras resulted in DNA duplication and hyperphosphorylated and unusually folded Tau proteins, similar to those observed in Alzheimer's disease. However, the disrupted DNA repair mechanisms does not adequately explain the formation of Aβ plaques, and as before there is little support for this hypothesis from genome wide association studies.

The observation that the Alzheimer's disease brain has a much-reduced capillary and vascular network has led to the formulation of the vascular hypothesis. There is much evidence linking the brain's vascular network and the disease.

Research has found that hypertension and diabetes, which both have vascular effects, significantly increase the risk of the development of the disease (Prince et al., 2014). In one study, it was discovered that the formation of 85% of the amyloid plaques in AD brains were either centered or proximal to vasculature (Kumar & Singh et al., 2005). A study of several different forms of dementia including AD revealed substantial reductions in microvasculature (Buee et al., 1994). Although there appears to be a credible link between the disease and the vasculature, it is not entirely clear if AD causes damage to the vasculature or if a vascular insufficiency promotes deposition of Aβ plaque (Karran & De Strooper, 2016).

One final hypothesis to consider is the Aβ oligomer hypothesis (AβOH). This theory is a variation of the original ACH. The main traction behind this theory is that it provides possible explanation as to why the density and amount of amyloid plaques does not correlate with the symptoms of AD. According to the AβOH the oligomers act at a distance from the plaques and mediate their effects. There is a large amount of data in support of this view. These include the treatment of cells with Aβ oligomers to induce neuronal death, the impact of the oligomers on insulin and nicotinic receptors, and the injection of these oligomers in rat brains which induced impaired memory and cognition. The AβOH would also explain why amyloidocentric drugs have had little success (Karran & De Strooper, 2016).

The cholinergic hypothesis and the amyloid cascade hypothesis clearly are supported by the strongest evidence. The earliest experiments demonstrating the reduction in cholinergic function provided the basis for much of the later research into Alzheimer's disease. Studies have consistently shown reductions in the levels of enzymes responsible for acetylcholine synthesis, and loss of receptor activity disrupting its uptake into neurons. This together with the degeneration of cholinergic neurons has provided the basis for this hypothesis. The genetic studies into familial Alzheimer's disease and the link between the disease and Down's syndrome laid the path for the formulation of the ACH which is the most comprehensive hypothesis in Alzheimer's disease. The cholinergic hypothesis fails to explain the mutations in APP in familial Alzheimer's disease, the formation of the Aβ peptide, amyloid plaques and neurofibrillary tangles. The Amyloid cascade hypothesis too has its inconsistencies and unanswered questions. However, rather than rejecting the undeniable data in support of each of these hypothesis, understanding the links and interactions between the two may provide a clearer picture of the mechanism of disease and provide a target for more effective therapy.

The connection between the cholinergic system and APP processing has been established. In one study, human embryonic kidney (HEK) cell lines were transfected with muscarinic acetylcholine receptors. As mentioned earlier there are five isoforms of muscarinic receptors; M1, M2, M3, M4 and M5. Cells transfected with M1 and M3 receptors showed significant increase in the release of APPα the product in the non-amyloidogenic pathway, when exposed to Carbachol a muscarinic receptor agonist. These cells also released lower levels of Aβ (Nitsch et al., 1992). Similar effects were seen in rat cerebral cortex slices that were exposed to M1 receptor agonists (Pittel et al., 1996). In addition to the effect the cholinergic system has on APP processing, Aβ peptides appear to modulate cholinergic function. Picomolar and nanomolar concentration of the beta amyloid peptide inhibit acetylcholine release from neurons in studies conducted on rat hippocampus and cortex sections. The exact mechanism by which the Aβ adversely effects acetylcholine release remains unclear. However, it appears that the Aβ peptides inhibit uptake of choline in the presynaptic neurons. The activity of choline acetyltransferase (ChAT) appears to be unaffected, while high affinity choline uptake is decreased by 20 minutes after incubation with Aβ (Kar et al., 2004). Further studies on rat septal neurons found that Aβ42 reduces the levels of acetylcholine by inhibiting the activity of the enzyme pyruvate dehydrogenase (PDH). The inhibition of pyruvate dehydrogenase results in a reduction in the biosynthesis of Acetyl-CoA, which is critical for acetylcholine synthesis. Aβ peptides have also been shown to disrupt cellular signaling and secondary messengers activated by muscarinic receptors.

Aside from the disruption to the various cellular processes Aβ exposure is also toxic to neurons in the long term. Differentiated cholinergic cell lines were the most susceptible to the toxic effects of beta amyloid, while GABAergic and serotonergic cells were more resistant. As stated above Aβ peptide appears to effect choline uptake in neurons. Under conditions of choline depletion, cells synthesize acetylcholine using membrane phosphatidylcholine. Therefore, a severe choline depletion may result in regulated membrane turnover being disrupted and eventually lead to cell death.

Aβ has also been shown to have a role in the hyper-phosphorylation of Tau. Hyper- phosphorylated Tau can no longer associate with microtubules, effecting both cell structure and vital mechanisms of transport. In cholinergic cell lines, aggregated Aβ peptide induced the phosphorylation of Tau proteins. The exact mechanism is unclear but it is most likely a result of an increase in kinase activity as Aβ peptides have been shown to increase the activity of both MAP kinase and GSK3β.

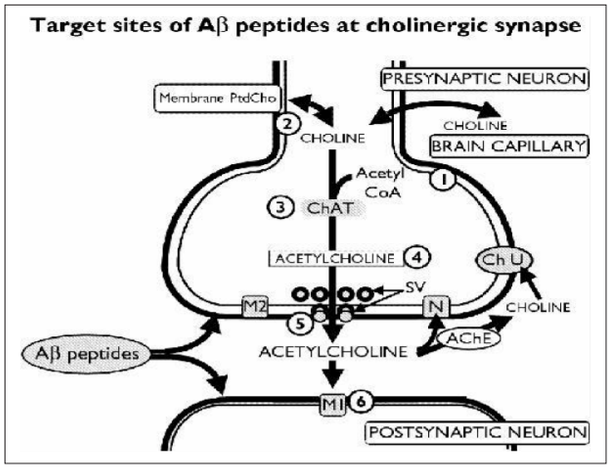

Figure 5 Targets of Aβ that modulate cholinergic transmission: (1) Aβ reduces activity of pyruvate dehydrogenase, an enzyme that generates acetyl coenzyme A (CoA) from pyruvate; (2) Aβ reduces high-affinity uptake of choline; (3) long-term or high-dose exposure to Aβ reduces activity of the choline acetyltransferase (ChAT) enzyme; (4) Aβ reduces acetylcholine (ACh) content; (5) Aβ reduces ACh release from synaptic vesicles (SV); (6) Aβ impairs muscarinic M1-like signalling. AChE = acetylcholinesterase, Ch U = site of choline uptake, M2 = presynaptic muscarinic M2 receptor, N = presynaptic nicotinic receptor, PtdCho = phosphatidylcholine. (Kar et al., 2004).

Conclusion

With the wealth of evidence in support of both the cholinergic hypothesis and the amyloid cascade hypothesis, an attempt at fully understanding the mechanism of disease will require better understanding of the link between Aβ peptides and the cholinergic system. The cholinergic system has been shown to play a significant role in the regulation of APP processing. Reciprocally, studies have found that the accumulation of Aβ peptides disrupts the muscarinic and nicotinic receptors on cholinergic neurons while also having a downstream effect on the signaling pathway and secondary messengers. In addition, Aβ inhibits synthesis of acetylcholine in a variety of ways and cholinergic neurons are particularly susceptible to the toxic effects of Aβ peptide. Genetics provides the basis of support for the amyloid cascade hypothesis, and while the amyloid plaque density may not correlate well with cognitive impairment in Alzheimer's disease, its interactions with the cholinergic system may be responsible for the symptoms of AD. The exact mechanism of that interaction needs to be better elucidated, as that understanding provides the greatest potential for more effective and targeted therapy.

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Possible Causes of Alzheimer's Disease Related Amyloid-β Plaques and Neurofibrillary Tangles

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Abstract
Alzheimer's disease is a major cause of dementia in the elderly and is a global health concern. However, researchers are not sure what causes the characteristic amyloid-β plaque accumulation and neurofibrillary tangles in the brain. Several model mechanisms have been proposed to answer this question. This paper examines three of these possibilities. Research suggests that a particular allele of the apoE gene is responsible for the neurodegeneration found in Alzheimer's disease. Another hypothesis is that the mechanism of Alzheimer's is related to prion-mediated protein misfolding. Other studies indicate that certain environmental factors can cause the neuropathology of Alzheimer's. Specifically, this paper will investigate the effects of a neurotoxin produced by cyanobacteria. Each of these possibilities is backed by supporting evidence, and there is probably not just one cause. Alzheimer's is a complex disease caused by a combination of interacting factors that may include these models, as well as others that have not been focused on in this paper. The more that is discovered about the multiple possible causes of Alzheimer's, the closer we are to developing ways of preventing these pathways in the hope of a cure.

Introduction
Alzheimer's disease is the most common cause of dementia in the elderly (Seeley, Miller 2015) and a devastating disease for those affected and their families. According to the Alzheimer's Association, Alzheimer's disease affected 5.4 million American people in 2016. Age is a major risk factor for developing Alzheimer's, and the risk increases the longer a person lives past age 60. A person over the age of 65 has a one in nine chance of having the disease. These numbers will only increase in the future years. Because of advancing science and better treatments, people are living longer, therefore increasing the chances of developing this disease. The average cost of caring for a person with Alzheimer's is over $50,000 a year, creating an emotional and financial burden on caregivers and family members (Seeley, Miller 2015).

Alzheimer's disease progresses in stages, and the first symptoms noted are generally memory related. The disease is usually diagnosed in the "mild" stage, where memory loss becomes more apparent and the patient exhibits other cognitive impairments (NIH). It is important to note that these are not symptoms of normal aging; this is evidence of a disease state in the brain (CDC). As the disease progresses to moderate and severe Alzheimer's, the patient gradually loses language ability, reasoning, sensory processing and conscious thought. The disease culminates in the patient being completely uncommunicative and bedridden, wholly dependent on a caregiver (NIH). The disease usually lasts for 8-10 years and ends in death from secondary factors, such as malnutrition or pneumonia. This pattern of degeneration is patterned by the atrophy of brain tissue, which begins in the medial temporal lobes, then affecting the lateral parietal, medial parietal and temporal lobes before spreading to the lateral frontal cortex, as seen by brain imaging of Alzheimer patients (Seeley, Miller 2015).

The brain tissue of an Alzheimer patient exhibits significant shrinkage and characteristic amyloid plaques and neurofibrillary tangles (Seeley, Miller 2015). Amyloid plaques consist of beta-amyloid oligomers that stick together. These beta-amyloid fragments arise from a transmembrane protein called amyloid precursor protein (APP). Different enzymes called alpha-secretase, beta-secretase, and gamma-secretase can cleave the APP protein in different places causing different results. In a healthy pathway, alpha-secretase will cleave off a fragment called sAPPα, which may be beneficial to the neuron. The rest of the APP fragment is cleaved with gamma-secretase and released. In the harmful pathway, APP is cleaved with beta-secretase which releases sAPPβ. The residual fragment is cleaved with gamma-secretase and is released as amyloid-β. These amyloid-β fragments accumulate and become the damaging amyloid plaques found in Alzheimer patients (NIH).

Another feature of Alzheimer's disease is the neurofibrillary tangles caused by the protein tau, which normally helps support the internal structure of the neurons. Neurons contain microtubules, which support them and help in transporting nutrients. Attached to these microtubules are the tau proteins that aid in stabilization. In Alzheimer's the tau proteins are hyperphosphorylated, which causes the tau proteins to separate from the microtubules and tangle together with other tau threads. The microtubules break down which causes the neuron's transport system to fail, compromising the neuron's ability to communicate (NIH).

These misfolded proteins cause the degradation of neurons in Alzheimer's and the subsequent brain atrophy. The causes and possible treatments for the accumulation of beta-amyloid and the neurofibrillary tangles are the subjects of ongoing research. This paper explores three different current ideas as to what causes these plaques and tangles to form, and by what mechanism they cause disease.

Methods
Articles and studies researched in this paper were obtained through the Ebsco and AccessMedicine databases with access provided by Touro College Library. Pubmed and Google Scholar were used to find other research articles. Several papers were obtained by use of the Touro College interlibrary loan system. Some of the research articles were kindly provided by Dr. Zev

Leifer, who has an interest in the topic. In addition, references in these articles were used as additional sources of information.

The Effect of apoE on the Development of Senile Plaques

Several possible causes of Alzheimer's disease are currently under investigation. Recent research has been done to study genetic factors of the disease. It has been found that the apolipoprotein E (apoE) gene exists in several alleles, and one, in particular, contributes to the biochemical mechanism of Alzheimer's disease (Liraz et al., 2013). Alzheimer's disease is characterized by the formation of senile plaques composed of amyloid-β, a 40-42 amino acid long peptide derived from amyloid precursor protein. Another feature is the neurofibrillary tangles that arise from the hyperphosphorylated protein tau, which leads to a compromised cytoskeleton and cell death. The apoE gene encodes for the apoE protein, which is produced by astrocytes in the brain. It is an important cholesterol carrier which is involved in the transport of lipids and injury repair. ApoE exists as three alleles: apoE2, apoE3, and apoE4. ApoE is a 299 amino acid protein, and the difference between the isoforms is at amino acid 112 and 158, where either a cysteine or arginine is present (Lui et al., 2013). This slight difference is enough to cause a change in the protein's tertiary structure, which affects its ability to bind lipids, receptors, and amyloid-β. ApoE3 is the most common allele, and apoE2 is associated with a decreased risk of AD (Simonovitch, et al., 2016). ApoE4 is a hypolipidated version of the protein, and it has been linked to an increased risk of developing Alzheimer's. A greater percentage of the apoE4 allele has been noted in families with late-onset and sporadic Alzheimer's. In sporadic AD, the frequency of apoE4 is greater than 50%, and each copy of the allele decreases the disease onset 7-9 years. Patients with a higher percentage of apoE4 have been found to have a higher level accumulated amyloid-β and hyperphosphorylated tau, as well as a decreased neural plasticity and neuropathology (Liraz et al., 2013). The fact that amyloid-β accumulation is increased in apoE4 positive patients led researchers to believe that the two pathways interact with each other, and cross-talk between them causes the pathological effects. The presence of the apoE4 allele adversely affects the binding of apoE4 to lipids, leading to the proposal that the disease is caused by lipid-related mechanisms (Liraz et al., 2013).

Impaired Autophagy in Alzheimer's Disease

Additional research on how apoE4 is related to Alzheimer's disease has shown how other factors can also play a role in the disease process. Besides causing the formation of senile plaques, the apoE4 protein can also impede the body's means of removing it. Amyloid plaques and neurofibrillary tangles are usually removed from the brain through the process of autophagy (Simonovitch et al., 2016, p. 917). Autophagy clears out cellular debris such as dysfunctional organelles, old proteins, and aggregated proteins. Normally astrocytes produce apoE in the central nervous system, which protects the brain from harmful protein buildup. However, the mechanism becomes faulty as the disease progresses, and impaired autophagy may become harmful because the accumulated protein can cause synaptic degeneration in hippocampal neurons. In 2016, an experiment was done to connect the presence of the apoE4 gene to the accumulation of amyloid-β buildup by the process of decreased autophagy. This experiment used knock-in mice with either apoE3 or apoE4 human cell lines and 5XFAD mice to replicate the disease process. 5XFAD mice have a phenotype similar to Alzheimer disease and exhibit many comparable characteristics such as amyloid plaques (Alzforum). Astrocytes were isolated from the apoE3 and apoE4 mice and cultured with frozen coronal brain sections from the 5XFAD mice. The experimental results were based on the ability of the astrocytes to clear the amyloid-β plaques from the brain slices. Brain slices with no astrocytes were used as a control. Amino acid deprivation was used to induce autophagy, and results were measured at the first, second and fourth hours of starvation. Results were calculated by means of the LC3 and p62/SQSTM1 proteins. LC3-I becomes a lipidated form, LC3-II, during autophagy and becomes associated with the autophagosomal membrane. P62 is a protein that is associated with LC3 and is degraded during autophagy. By looking at the LC3-II/LC3-I ratio, the rate of activation of autophagy can be monitored and measured. A higher ratio indicates greater autophagic activation, which in turn indicates more autophagy and more clearing of the amyloid plaques. This ratio was significantly higher in the apoE3 astrocytes compared to the apoE4 astrocytes. In addition, the levels of p62 decreased more rapidly in the apoE3 astrocytes compared to the apoE4 astrocytes following starvation. This evidence suggests that the initiation of autophagy in apoE4 astrocytes is somehow impaired in Alzheimer's disease (Simonovitch et al., 2016).

To test this hypothesis, the researchers examined the autophagy levels of the astrocytes when an autophagy inducer and inhibitor were added in separate experiments. Rapamycin was used as an autophagy inducer, and chloroquine was used as an inhibitor. In these sets of experiments, the apoE3 and apoE4 astrocytes were treated with rapamycin and chloroquine in separate trials. The results were measured with the LC3-II/LC3-I ratio and the rate of p62 degradation. The results showed that with treatment with rapamycin, the apoE3 cells were even more effective at clearing the amyloid-β plaques, and while it was more effective than the apoE4, the apoE4 was still more effective than the non-treated apoE4 cells. In contrast, treatment with chloroquine entirely prevented autophagy in both apoE3 and apoE4 cells (Simonovitch et al., 2016) This research highlights how the apoE gene can be connected to the buildup of the amyloid-β

plaques in Alzheimer's disease. It demonstrates that the presence of the apoE4 allele affects the body's mechanisms of naturally clearing protein buildup through the process of autophagy and that the problem is probably at the beginning of the process when autophagy is first induced. This research shows that not only is Alzheimer's disease caused by the buildup of misfolded proteins but also that the body's normal mechanism of clearing it away is also adversely affected.

ABCA1 Lipidates ApoE

Knowing how the function of apoE4 is different from apoE3 is valuable for possible therapeutic strategies. If apoE4 can be made to function like apoE3, many of the symptoms and pathologies of Alzheimer's disease can potentially be reversed. Although the full pathological process of apoE4 is not yet fully understood, evidence suggests that it is connected to lipid-related mechanisms (Boehm-Cagan et al., 2016). ApoE lipidation is controlled by the ATP-binding cassette transporters ABCA1 and ABCG1. ABCA1 triggers the efflux of cholesterol and phospholipids onto the apoE acceptor, and ABCG1 has a similar function. Studies have shown that apoE4 is less lipidated than apoE3 and is less effective at stimulating the efflux of cholesterol and phospholipids in a cell culture. These proteins are regulated by the retinoid X receptor transcription regulating system. If this system can be activated by some means, the pathological effects of apoE4 may be alleviated.

CS-6253 Can Activate ABCA1

CS-6253 is a non-toxic peptide derived from the carboxyl terminal of apoE (Boehm-Cagan et al., 2016). It has been shown to interact with and stimulate ABCA1 to activate cholesterol and phospholipid efflux. A study was done to see what effect CS-6253 has in vivo on the neuropathology and cognitive decline in apoE4 mice. ApoE3 and apoE4 mice were injected intraperitoneally with CS-6253 every 48 hours for 6 weeks. A control group was injected with saline. They were then subjected to a series of cognitive tests to measure learning and memory. During the novel object recognition test, mice were placed in an arena with no objects. Then, two objects were introduced. 24 hours later the mice were replaced in the arena with one familiar object, and one new one. Their behavior and interactions with the objects were monitored and measured. During the Morris maze test, the mice were placed in a circular pool of cloudy water with a submerged hidden platform. The test examined the mice's memory by measuring the time it took the mice to reach the platform after being placed in the pool previously. After the experiment, the mice were euthanized and the brains were removed to be stained and studied (Boehm-Cagan et al., 2016).

The results of this experiment showed many important findings on the effect of CS-6253. Treatment with CS-6253 significantly lowered the buildup of amyloid-β in the test apoE4 mice compared to the untreated mice. The levels of amyloid-β in the treated apoE4 mice brains became similar to the untreated apoE3 mice, who were unaffected by the treatment. Treatment with CS-6253 also had an effect on the buildup of phosphorylated tau. The tested apoE4 mice showed a marked decrease of phosphorylated tau compared to the untreated apoE4 mice. Injections of CS-6253 also affected the results of the cognitive tests. Before treatment, apoE4 mice took a significantly longer time to reach the platform in the Morris maze test compared to the apoE3 mice. After treatment, the performance of the treated apoE4 mice was similar to the apoE3 mice. The treated apoE4 mice also scored similarly to the apo3 mice during the novel item recognition test after they were treated with CS-6253. The experimental findings show that CS-6253 can successfully activate ABCA1 to reverse the hypolipidation of apoE4, decrease the levels of accumulated amyloid-β and phosphorylated tau, and reverse the cognitive deficiencies of apoE4 mice. These results indicate that ABCA1 can be an important therapeutic target in treating Alzheimer's disease (Boehm-Cagan et al., 2016).

Alzheimer's Nay be Linked to Prion Diseases

Many characteristics of Alzheimer's disease are similar to prion diseases, suggesting that there may be a connection between the two (Castellani et al., 2004). Prion diseases are characterized by the buildup of misfolded prion proteins (PrP) that can propagate themselves by causing other healthy protein to misfold in the same way (Prion Alliance). Prion diseases can affect humans and animals. The five human prion diseases are Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, fatal familial and sporadic insomnia, kuru, and new variant Creutzfeldt-Jakob disease. Alzheimer's disease is similar to these prion diseases in that both have an age requirement for the symptoms to become apparent, both usually occur sporadically although there is a genetic component in some cases. Most prion diseases appear in middle age to older patients, and that is the age demographic that is affected by Alzheimer's. There is significant overlap between the two diseases' characteristics. In both diseases, there is allelic segregation, with an allele that makes a person susceptible to getting the disease. In prion disease, this gene is the PRNP gene, and in Alzheimer's disease, it is ApoE and presenilin 1 genes. Furthermore, in a certain type of Gerstmann-Straussler-Scheinker syndrome, prion plaques in the gray matter also have tau protein with neurofibrillary tangles, amyloid-β accumulation, and gradual memory loss over a longer span of time than typical prion diseases, more similar to the pattern seen in Alzheimer's disease (Castellani et al., 2004).

The word prion comes from "proteinaceous infectious particle" (Jeffrey 2013). These particles are neither bacteria nor virus, but they cause transmissible diseases. Prion diseases are also known as "transmissible spongiform encephalopathies" because they are neurodegenerative and ultimately fatal. Amyloid plaques are frequently present in these diseases, which leads to the possibility that Alzheimer's disease may be related. Prions are caused by a pathogenic form of cellular prion proteins (PrPc), which are encoded by the host's PRNP/prnp gene (Jeffrey 2013). PrPC is an endogenous, cell surface glycoprotein of unknown function (Elezgarei, Biasini 2016). In prion diseases, this normal protein undergoes a conformational change and is called a "scrapie form of PrP," or PrPSc. This misfolded isomer accumulates in the central nervous system of affected individuals. PrPSc, or prions, are capable of propagating themselves by binding to normal PrPC proteins and forcing their conformational change to new PrPSc, thereby perpetuating a cycle that leads to a buildup of misfolded proteins, which causes neurodegeneration and cell death (Elezgarei, Biasini 2016). PrPSc changes PrPC into prions by affecting the tertiary structure of the protein. Normal PrP is made up of 209 amino acids and the tertiary structure is 40% alpha helix with minimal beta sheet. PrPSc however, is made of 40% beta sheet and 30% alpha helix (Castellani et al., 2004). Research shows that the prions are formed when alpha helixes are converted into beta sheets. This change causes PrPSc to be insoluble and resistant to proteolytic enzymes (Castenelli et al., 2004).

Similarities Between Amyloid-β and Prions

Amyloid-β oligomers in Alzheimer's disease have similarities to prions. Soluble amyloid-β oligomers affect the neuronal synapse in Alzheimer's disease, causing synaptic abnormalities and neurodegeneration (Elezgarei, Biasini 2016). These oligomers are different from the insoluble amyloid-β that builds up in the brain. There is evidence that the amyloid-β oligomers bind to PrPC in a similar manner to prions, and cause a cascade that leads to cell death (Laurén et al., 2009). PrPC can act as a receptor site on the cell membrane of neurons, which have been shown to bind amyloid-β and mediate the pathological effect. Suppression of these receptors may, in fact, have therapeutic benefit for people suffering from the disease (Laurén et al., 2009) PrPC-amyloid-β complexes occur in the brains of Alzheimer patients, which is further evidence that this prion-like mechanism is implicated in Alzheimer's disease. PrPC is not necessarily involved in all cases of Alzheimer's; Amyloid-β can cause neural damage without PrPC (Elezgarei, Biasini 2016). This fact demonstrates the complexity of the disease and that the cause may be due to several factors. There are several similarities between amyloid-β precursor protein (AβPP) in Alzheimer's disease and PRNP in prion diseases (Castenelli et al., 2004). Mutations in both of these genes can cause a buildup of their respective proteins with increased beta sheet structure.

Disease Transmission

Classic prion diseases are transmissible from one affected individual to another. They are able to be passed from person to person, from animal to person, and from person to animal. PrPSc initially forms in sporadic diseases by nucleation. The initial formation of the protein is kinetically less favorable; however, once it overcomes the activation barrier it is able to propagate, changing PrPC to PrPSc very easily (Beekes et al., 2014). Once you have an initial prion "seed," it can quickly proliferate and corrupt other proteins (Figure 1). However, prions are not infectious in the typical sense; you cannot "catch" a prion disease through direct contact (Weissmann et al., 2002). Transmission of prions occurs perorally and perenterally. The kuru epidemic that occurred in Papua New Guinea in the 1900's was spread through cannibalism. A greater concern is the spread of prions to humans iatrogenically. Prion contaminated cadaver-derived human growth hormone gonadotrophin has caused Creutzfeldt-Jakob disease in people in the past, and surgical instruments that have been used on affected people have caused the disease in others, even after being properly sterilized. If Alzheimer disease is so similar to prion diseases, can Alzheimer's be spread through such means? Studies were done where amyloid-β taken from the brain tissue of Alzheimer's patients was injected into mice (Kane et al., 2000). This resulted in the stimulation of amyloid-β deposition in mice that were genetically predisposed to the formation of such plaques. Amyloid-β deposition was recovered past the site of injection, similar to prion spreading. Inoculation of amyloid-β in wild-type mice initiated amyloid-β build up in brain tissue at the site of injection, and in certain species, in distant areas of the brain as well. However, none of the mice developed cerebral neurodegeneration or cognitive decline (Beekes et al., 2014). Similar experiments were done with the protein tau (Sanders et al., 2014). The protein was able to replicate itself with high fidelity in a prion-like manner and cause an increase of hyperphosphorylated tau in the mouse brain tissue. Nevertheless, this buildup of tau did not cause cognitive decline or severe disease in these mice. Epidemiological studies investigated the transmission of Alzheimer's disease between humans in real life (Beekes et al., 2014). Available data indicates that Alzheimer's disease is not likely to be spread between people. Blood transfusions do not cause a risk of Alzheimer's in the recipient, and neither does plasma protein therapy for hemophilia. Another study examined the risk associated with receiving cadaveric human growth hormone, derived from the pituitary gland of the deceased. Such growth hormone has caused over 200 cases of iatrogenic Creutzfeldt-Jakob disease because of

PcPSc seeds that contaminated the hormone and caused the prion disease in the recipient. In this study, researchers found small amounts of amyloid-β in the pituitary glands of the recipient. Yet, none of the recipients studied developed Alzheimer's disease. Based on these studies, as similar as Alzheimer's disease is to prion diseases, there is an essential difference when it comes to disease transmission. Although seeding effects of Amyloid-β can somewhat cause neurotoxicity, it does not cause the neurodegenerative a cognitive impairment characteristic of Alzheimer's (Beekes et al., 2014).

Environmental Effects on Neurodegenerative Disease: Mystery Disease in Guam is Similar to Alzheimer's

The environment can have a role in the development of neurodegenerative diseases such as Alzheimer's. On the Island of Guam, many of the native villagers, known as Chamorros, suffer from a debilitating neurodegenerative disease with symptoms similar to Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Parkinson's disease (Cox et al., 2016). The illness caused paralysis, shaking, and dementia at 50-100 times the incidence of ALS worldwide (Holtcamp 2012). This disease was first described in the 1950's by US Army physicians, who called it amyotrophic lateral sclerosis/Parkinsonism dementia complex (ALS/PDC). Amyloid-β plaques and neurofibrillary tangles were present in the brains of Chamorros who developed this mystery disease. Although in some villages a quarter of adults died from this disease, no distinct pattern of heredity was apparent. Because outsiders who adopted the Chamorro lifestyle were also affected, the cause of this disease seemed likely to be an environmental toxin (Cox et al., 2016).

The BMAA Hypothesis

There was difficulty in determining the exact nature of the toxin because symptoms could appear years after the exposure. However, researchers were able to isolate the neurotox-

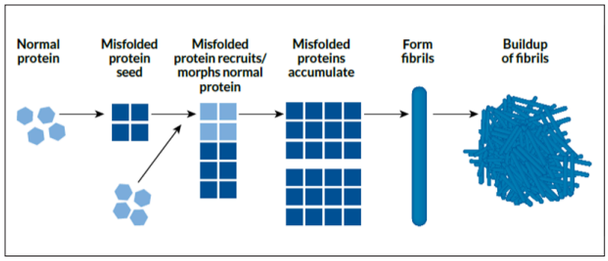

Figure 1: Mechanism of prion mediated misfolding of protein. (Sanders 2015

in β-N-methylamino-L-alanine (BMAA) from the cycad seed, which the Chamorros use to make flour, a staple in their diet. In the 1980's, BMAA was fed to macaques which caused acute neurological symptoms. However, these results were disregarded when it was shown that a human would have to consume an inordinate amount of cycad seed flour to ingest a comparable dose (Cox et al., 2016). Paul Alan Cox, an ethnobotanist, arrived in Guam in the 1990's and revived the hypothesis that BMAA causes ALS/PDC seen in the natives (Holtcamp 2012). With his colleague Sandra Banack, he was able to show that BMAA was produced by a symbiotic cyanobacteria living in specialized cycad roots. BMAA accumulates in the gametophytes of the cycad seeds that are ground and eaten by the villagers. Cox then showed that animals such as flying foxes, feral deer, and pigs all feed on the cycad seeds, which are then eaten by the Chamorro people. BMAA is biomagnified in these mammals 10, 000 times that which is produced by the cyanobacteria. This process of biomagnification allows a sufficiently toxic amount of BMAA to accumulate in the food chain that it can cause neurological symptoms in the Chamorro people. This problem is not limited to Guam. BMAA producing cyanobacteria live in many marine ecosystems that biomagnify BMAA up the food chain, accumulating in sharks, bottom-dwelling fish, and shellfish (Cox et al., 2016). People who eat these organisms are at risk of BMAA ending up in their brain tissue.

Mechanism of Toxicity of BMAA

BMAA is readily able to cross the blood-brain barrier into the brain and central nervous system. It is mistaken for L-serine by the cellular mechanism and misincorporated into proteins, leading to misfolding, aggregation, and apoptosis of the cell (Cox et al., 2016). In normal proteins, the hydrophilic part is on the outside of the structure and the hydrophobic part is on the inside. However, because of protein misfolding due to the incorporation of BMAA, the hydrophobic part can end up on the outside, exposed. These hydrophobic areas are sticky and tend to clump together forming the characteristic aggregates of neurodegenerative plaques. These small aggregates end up forming larger ones that prevent the cells from functioning properly (Holtcamp 2012). A misincorporation rate of even 1 in 10,000 has been shown to have a neurodegenerative effect in laboratory animals. BMAA can also result in phosphorylated tau by activating mGluR5, a metabotropic glutamate receptor, which decreases the activity of protein phosphatase 2A (PP2A). PP2A is significantly decreased in Chamorro ALS/PDC brains, resulting in an increase of phosphorylated tau (Cox et al., 2015).

Neuropathology Caused by BMAA in an Animal Model

To further prove that BMAA is responsible for the neurodegenerative disease, Cox and his colleagues conducted an experiment in which they attempted to show that chronic exposure to BMAA causes neuropathology and that BMAA can be re-isolated from the induced organisms. Vervets, a type

of non-human primate, were the subject of this study. The vervets in this experiment were exposed to L-BMAA in their diets for 140 days, and then the brain tissue was examined for tau and amyloid deposition. Sixteen juvenile vervets divided into 4 cohorts of 4 were used in this experiment. The first cohort of vervets was given a piece of fruit dosed with 651 mg of L-BMAA. Because L-serine has been shown to prevent misincorporation of BMAA into proteins, a cohort was given was given 651mg of L-BMAA and 651 mg of L-serine. A third cohort was given just 651 mg of L-serine. Finally, a control group was given a piece of fruit with 651 mg of rice flour as a placebo. A second experiment was done using adult vervets, also using four cohorts. The first cohort was given 987 mg of L-BMAA. The second was given 98.7 mg of L-BMAA, a tenfold reduction. The third was given 987 mg of L-BMAA, and 987 mg of L-serine. The fourth, a control, had 987 mg of rice flour as a placebo (Cox et al., 2016).

In the first experiment, the vervets that received L-BMAA had neurofibrillary tangles and sparse amyloid-β plaque buildup in many areas of the brain. In contrast, the cohort that received L-serine and the control cohort did not show signs of amyloid-β or tau inclusions. The cohort that received an equal amount of L-BMAA and L-serine showed an 80-90% reduction in amyloid-β plaques and neurofibrillary tangles. In the second experiment, all BMAA exposed vervets developed hyperphosphorylated tau proteins and neurofibrillary tangles. The density of the neurofibrillary tangles in the brain was clearly related to the dose of BMAA administered. Besides for the control cohort, the low dose cohort had the lowest median count of neurofibrillary tangle density; the median density of the high dose cohort was more than twice the levels of the low dose cohort. The cohort that received both high dose BMAA and L-serine had a 50% decrease of neurofibrillary tangles compared to the high dose cohort. Exposure to BMAA also increased the chances of developing amyloid-β deposits, which were not found in any of the control vervets (Cox et al., 2016).

This study shows that dietary exposure to BMAA can cause the formation of neurofibrillary tangles and deposition of amyloid-β (Figure 2). BMAA-producing cyanobacteria live worldwide and can have an important effect on human health. BMAA may possibly act as an initiator for sporadic Alzheimer's disease by triggering the formation of amyloid plaques and neurofibrillary tangles. This study also indicates that L-serine is able to reduce the risk of formation of the characteristic plaques and tangles in the brains tissue of affected individuals, and that L-serine may have a possible therapeutic role in the treatment of mild cognitive impairment and early Alzheimer's disease (Cox et al., 2016).

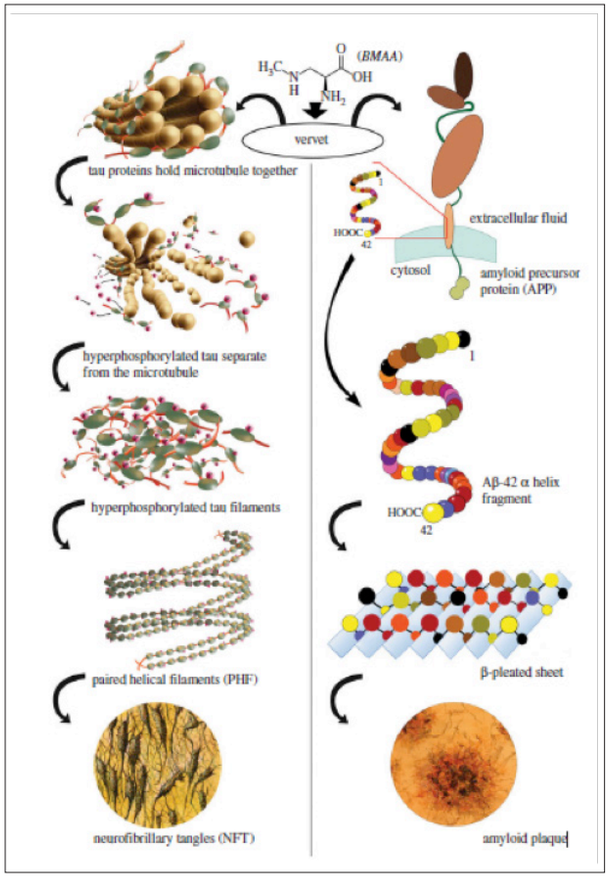

Figure 2: Theoretical pathways of development of ALS/PDC and Alzheimer's disease neuropathology from dietary BMAA exposure causing neurofibrillary tangles and amyloid-β plaques. (Cox et al.,2016)

Conclusion

This paper investigated three seemingly unrelated mechanisms that have been proposed to cause the neuropathology seen in Alzheimer's disease. Although the means of each method reviewed are very different, they have all been studied as a potential cause of the buildup of the characteristic plaques and tangles present in the disease. Alzheimer's disease is a public health problem; it is the sixth-leading cause of death in the United Stated, and fifth among people 65 and older (Alzheimer's Association). Therefore it is important to study various models of the mechanism of the disease in the hope of gaining a better understanding of it, and eventually finding a cure. These three models may seem disconnected from each other, but in reality, Alzheimer's disease is a complex disease. There are multiple factors involved that cause a person to develop Alzheimer's related neurodegeneration; there is no one "correct" model. The three mechanisms discussed are only part of the multipart picture, and yet they are important to understand to get a complete understanding of the various pathways that lead to this disease.

By examining different routes, hopefully some light will be shed on questions like; why, if two people have a genetic predisposition, does one develop Alzheimer's and one does not? Only by studying the causes will we ever discover a cure to the disease that affects so many.

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Repurposing Diabetes Drugs to Treat Insulin Resistance in Alzheimer's Disease

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Abstract

Alzheimer's disease is a neurodegenerative condition which results in a significant decline in cognitive status. Novel treatment approaches for Alzheimer's are sorely needed, as current medications for the disease offer only marginal clinical benefit. Research has discovered a connection between the pathology of Alzheimer's and Type 2 Diabetes, two serious and seemingly unrelated disorders. Clinical studies have shown that Alzheimer's disease is associated with brain insulin resistance similar to the pathology of Type 2 Diabetes. This observation has led to the notion that drugs developed for the treatment of Type 2 Diabetes may be beneficial in modifying the cognitive function and pathophysiology of individuals suffering from Alzheimer's disease. This paper offers a comprehensive review of the clinical studies demonstrating the potential of using diabetes medications as an effective therapeutic method for the prevention and treatment of Alzheimer's disease. Special focus is given towards the metabolic hormones insulin, amylin and leptin.

Introduction

Alzheimer's and Type 2 Diabetes are two of the most common diseases afflicting the elderly population today. A 2016 Report by the Alzheimer's Association found that of the 5.4 million Americans with Alzheimer's, an estimated 5.2 million people are age 65 and older (Alzheimer's Association, 2014). Similarly, the American Diabetes Association reported that in 2012, of the 29.1 million Americans with Diabetes, an estimated 11.8 million people were age 65 and older (American Diabetes Association, 2012). The two conditions have traditionally been treated as independent disorders. However, recent studies linking Alzheimer's to Type 2 Diabetes have led to the proposal that Alzheimer be referred to as "Type 3 Diabetes". Discoveries of common pathological mechanisms between these two diseases have given rise to novel clinical trials incorporating diabetes therapies to treat Alzheimer's disease.

Methods

A variety of literary reviews and research papers on the subject were collected through use of the PubMed database. Keywords such as Alzheimer's disease, insulin resistance and Type 2 Diabetes were used to search for relevant material. Access to PubMed was provided by the Touro College online library system.

Pathophysiology of Alzheimer's and Type 2 Diabetes

Alzheimer's disease is a neurodegenerative condition, which results in nerve cell death and tissue loss throughout the brain (Li, Z et al., 2015). Scans of brains of individuals suffering from Alzheimer's demonstrate severe shrinkage of the hippocampus and cerebral cortex, as well as the enlargement of the ventricles (Querfurth et al., 2010).

The pathophysiology of Alzheimer's disease is described by the amyloid cascade hypothesis. Cleavage of amyloid precursor protein (APP) leads to the formation of the protein amyloid beta. Excessive cleavage of APP coupled with inefficient removal of amyloid beta can lead to the formation of amyloid beta plaques in the brain. These plaques damage and destroy brain cells by blocking cell-to-cell signaling at synapses (Reitz, 2012). Amyloid beta plaques have also been shown to cause apoptosis. The presence of amyloid plaques in the brain generates the production of harmful oxidative free radicals which in turn activates the c-Jun N-terminal kinase (JNK) pathway. The JNK pathway stimulates the transcription of several key target genes, including the death inducer Fas ligand. The binding of Fas ligand to its receptor Fas then induces a cascade of events that lead to caspase activation and ultimately neuronal cell death. (Yoshiyuki et al., 2001).

The tau protein also plays an important role in Alzheimer's disease (Lasagna-Reeves CA et al., 2012). This protein is integral to maintenance of internal support and transport systems between brain cells. Hyperphosphorylation, which is the addition of phosphate to too many amino acids, leads to the collapse of tau proteins into twisted strands referred to as neurofibrillary tangles (Jack et al., 2013). Without the support of the tau protein, the transport system in the brain collapses. Consequently, essential nutrients are unable to reach brain cells and cell death ensues (Gong and Iqbal, 2008).

Diabetes is a metabolic disorder which develops when the body does not make enough insulin or is unable to use insulin effectively. (Yarchoan and Arnold, 2014). If left untreated, diabetes can cause many complications, including kidney failure, diabetic ketoacidosis, heart disease and stroke (Schnell et al., 2016). Type 2 Diabetes is characterized by an insulin resistant state which is most commonly caused by obesity (Smith and Kahn, 2016). In insulin resistance, muscle, fat, and liver cells do not respond properly to insulin and thus cannot easily absorb glucose from the bloodstream. As a result, the body needs higher levels of insulin to help glucose enter cells. The pancreatic beta cells initially increase their insulin output but fail over time to keep up with the body's increased demands for insulin. Type 2 diabetes develops when insulin production is inadequate to overcome insulin resistance, resulting in the drastic rise in blood glucose levels (Yarchoan and Arnold, 2014).

Discovery of Insulin Resistance in Alzheimer's Disease

Insulin receptors are widely distributed in brain regions known to be involved in memory function, including the hippocampus, cerebral cortex and cerebellum (Werther et al., 2015). Clinical studies have found evidence for central insulin resistance in Alzheimer's brains. Post-mortem studies of brain tissues from people with Alzheimer's disease have discovered extensive abnormalities in insulin and insulin-like growth factor signaling mechanisms in the brain (Steen et al., 2005). A study of post-mortem human hippocampus Alzheimer's tissue was done by exposing the tissue to different concentrations of insulin. This allowed researchers to study the activation of insulin pathways in the brain tissue of people with Alzheimer's compared to the brain tissue of those with normal cognitive function. In normal brains, activation of the insulin receptor initiated a signaling cascade which led to the production of many downstream insulin signaling proteins. The blunted insulin response observed in the Alzheimer's brain tissues was similar to the insulin resistance observed in Type 2 diabetes peripheral tissues (Talbot et al., 2012).

The formation of amyloid beta plaques has been implicated as a possible impetus for the removal of insulin receptors in brain cells. Studies of Alzheimer's brains have demonstrated that the binding of amyloid beta oligomers to hippocampal neurons triggers the removal of dendritic insulin receptor substrates from the outer membrane of the cell (Xie et al., 2002). These studies prove that elevated amyloid beta levels induce the removal of cell surface insulin receptors, thereby furthering insulin resistance.

A molecular model has been proposed to explain how the amyloid beta plaques found in Alzheimer's disease promote insulin resistance (Dineley et al., 2014). This model is based on the inflammatory pathway observed in Type 2 Diabetes, where inflammatory cytokines play an important role in establishing insulin resistance. Signaling from the inflammatory cytokine Tumor Necrosis Factor (TNF) stimulates JNK (Hoeks et al., 2012). Activation of the JNK pathway by TNF results in serine phosphorylation of the insulin receptor (Zhao et al., 2008). In order to be activated, phosphorylation of the insulin receptor must occur at a tyrosine residue. Addition of the phosphate group at the serine amino acid in individuals with Type 2 Diabetes results in inhibition of the insulin receptor (Bomfim et al., 2012).

In a similar fashion, inflammatory cytokines induced by the presence of amyloid beta in the brain can lead to insulin resistance in Alzheimer's. Recent work suggests that soluble misfolded amyloid beta can induce inflammatory cytokines through an inflammatory pathway known as NF-κB-inducing kinase (NIK). The resulting inflammatory state induces insulin resistance through feedback inhibition of the insulin receptor (Carrero et al., 2012). In this case the many signaling chemicals produced by the inflammatory pathway stop the activity of the insulin receptor (Talbot et al., 2012).

Insulin resistance may promote Alzheimer pathology through several mechanisms. Firstly, abnormal insulin signaling may promote amyloid beta and hyperphosphorylated tau formation through the kinase GSK-3. The activity of GSK-3 is normally regulated through inhibition by the protein AKT, which is an important kinase in the insulin signaling cascade referred to as the IRS-1 –AKT pathway (Yarchoan and Arnold, 2014). Therefore, dysfunctional insulin signaling caused by disturbances in the IRS-1 –AKT pathway leads to increased GSK-3 activity. Studies of GSK-3 found that its activity increases tau protein phosphorylation (Li, X et al., 2006) and that it is involved in amyloid beta production (Takashima, 2006).

Abnormal insulin signaling also interferes with memory and learning pathways. Insulin is a direct regulator of the ERK/MAP kinase pathway. This pathway is essential to the induction of longer term potentiation (LTP) and memory consolidation in the hippocampus (Winder 1999). LTP is responsible for the maintenance of long term memory, which is the ability to recall episodes which are not part of the immediate past (Kelleher et al., 2004). Disruptions of LTP and memory consolidation contribute to the impaired cognitive function typified by Alzheimer's disease (Dineley et al., 2014).

Discussion: New Role for Diabetes Drugs as a Treatment for Alzheimer's

Given the role of insulin resistance and deficiency in the pathogenesis of Alzheimer's disease, it is possible that a drug currently prescribed for Type 2 Diabetes may able also be useful for Alzheimer's. Various diabetes drugs are under study to test for their potential activity in Alzheimer's. This section provides an overview of the various clinical studies which assess the potential prospects of using the diabetes drugs insulin and amylin, as well as the weight loss hormone leptin, to treat Alzheimer's.

Insulin

The administration of exogenous insulin has predictably become a prime focus of the effort to treat insulin signaling dysfunction in Alzheimer's. Clinical trials have demonstrated that insulin administration acutely affects behavior and cognitive performance in both healthy individuals and those suffering from Alzheimer's. A 2001 study assessed the effects of peripherally administered insulin infusion in non-impaired individuals (Kern et al., 2001). Two groups were infused with insulin for 6 hours, one at a high rate and the other at a low rate. Any effects of the insulin administration on blood glucose concentrations were counteracted by constant glucose infusions. Comparison of the results

of the high rate insulin and low rate insulin treatment groups indicated an improvement in the cognitive function of the high rate insulin group. Subjects exposed to higher insulin infusion rates demonstrated changes in auditory-evoked potentials, had enhanced memory as evidenced by improved word recall and improved cognitive flexibility as measured by the Stroop test. Similar benefits of peripheral insulin administration on cognitive function were observed in individuals with Alzheimer's. In a 1999 study, Alzheimer's patients showed improved story recall and attention during insulin infusion relative to saline infusion (Craft et al.,2012).

Despite the results of these clinical trials, there are two main concerns that must be addressed with regards to utilizing peripheral insulin administration as a treatment for Alzheimer's. Firstly, peripherally administered insulin cannot bypass the blood brain barrier in order to enter the central nervous system (CNS) and is therefore unable to affect and improve brain function. Secondly, peripheral insulin infusions can induce hypoglycemia. Hypoglycemia is a condition characterized by abnormally low blood glucose which can lead to a seizure or unconsciousness if left untreated. Although hypoglycemia was mitigated by simultaneous glucose infusions in both studies involving peripheral insulin administration, this solution is highly impractical outside of a research setting (Yarchoan and Arnold, 2014).

In light of these concerns, intranasal insulin is considered to be the best method for insulin delivery in Alzheimer clinical trials. Insulin that is delivered nasally bypasses the blood brain barrier and is rapidly delivered into the cerebrospinal fluid from where it can easily enter the CNS (Mao et al., 2016). Additionally, because intranasal insulin is preferentially delivered to the CNS, it is possible to achieve clinically relevant insulin concentrations in the CNS without causing systemic hypoglycemia (Yarchoan and Arnold, 2014).

Pilot clinical trials using intranasal insulin have had successful results. A 2008 clinical trial reported that delivery of intranasal insulin for 21 days improved story recall, attention and caregiver-rated functional status in cognitively impaired subjects or individuals with Alzheimer's (Reger et al., 2008). In another clinical trial subjects given insulin spray over a placebo demonstrated improved delayed memory and cognitive function (Craft et al., 2012).

One study sought to illustrate the underlying mechanisms through which intranasal insulin ameliorates Alzheimer's pathology (Mao et al., 2016). APP/PS1 mice possessing the pathological features of Alzheimer's disease received intranasal insulin treatments for a total of 6 weeks, while a control group received saline treatments. Tissue samples were harvested from both groups upon the conclusion of the treatment period.

In order to determine whether intranasal insulin enhances brain insulin signaling, the respective levels of key components of the insulin signaling pathway, such as the insulin receptor beta-subunit (IRB) and protein B kinase (AKT), were measured through Western Blot analysis. Tissue from healthy wild type mice was also analyzed to serve as a reference marker of normal levels. The total levels of IRB and AKT were significantly decreased in the saline treated APP/PS1 mice compared with wild-type controls. However, the levels of IRB and AKT in the mice which received intranasal treatment were closer that of the wild-type mice, indicating that intranasal insulin treatment can partially protect APP/PS1 mice from brain insulin signaling deficits.

Intranasal insulin was also shown to reduce the activation of JNK in APP/PS1 mice. Activation of the JNK pathway results in serine phosphorylation of the insulin receptor and induces apoptosis. The level of phosphorylation of JNK was significantly increased in the hippocampus of saline-treated mice compared with wild-type controls, signifying that intranasal insulin reduces JNK activation.

Immunohistochemical analysis of the tissue samples measured the amounts of amyloid beta plaque deposits in the brains of the APP/PS1 mice. The number of amyloid plaques in the APP/PS1 insulin-treated mice was significantly reduced in both the hippocampus and cortex compared to the saline control group. It was also discovered that the area of amyloid beta plaques was significantly decreased in both the hippocampus and cortex of the insulin treated mice. Additionally, quantitative analysis revealed substantially reduced soluble amyloid beta oligomers in insulin-treated APP/PS1 mice. This revelation is especially significant considering that soluble amyloid beta oligomers are considered the most neurotoxic form of amyloid beta. Enhanced neurogenesis was also observed in the insulin-treated APP/PS1 mice. It was shown that intranasal insulin significantly increased levels of DCX, a marker of neurogenesis. The overall conclusions of the study were that intranasal insulin treatment improves cognitive deficits, ameliorates defective brain insulin signaling, strongly reduces amyloid beta plaque formation, inhibits JNK activation and promotes neurogenesis in APP/PS1 mice.

Despite the successful results observed in studies involving intranasal insulin treatment, there is concern that chronic hyperinsulinemic conditions in the brain may actually promote brain insulin resistance. Excessive exposure to insulin in mice has been shown to lead to abnormal phosphorylation of key components of the insulin signaling pathway, such as AKT and GSK-3, in a manner consistent with insulin resistance (Kim et al., 2011). It may therefore be beneficial to explore avenues of diabetes treatments which restore the byproducts of insulin signaling without directly affecting insulin levels.

Amylin

Amylin is a metabolic hormone which is co-secreted with insulin by pancreatic beta cells (Adler et al., 2014). Amylin's systemic effects in diabetic patients include the lowering of blood glucose levels through delayed gastric emptying, increased satiety, and decreased secretion of glucagon, which is an antagonist of insulin (Yarchoan and Arnold, 2014).

Amylin's signaling activity has the potential to alleviate the detrimental effects of insulin resistance in Alzheimer's disease. Amylin binds to independent receptors in the brain to activate signaling pathways that converge with insulin signaling. Amylin activates the production of the protein AKT, which is needed for the proper regulation of GSK-3, a protein which can lead to increased production of amyloid beta plaques and hyper phosphorylated tau (Moon et al., 2011). Amylin is also a known modulator of the ERK signaling cascade, a pathway significant in the maintenance of long term memory and memory consolidation (Adler et al., 2014). An advantage of using amylin as a medication is that it poses no risk of hyperinsulinemia, as it can activate the insulin pathway without interfering with the concentration of insulin in the body (Yarchoan and Arnold, 2014).

One study investigated the potential outcomes of using amylin as a treatment for Alzheimer's. The first part of the study compared plasma human amylin levels between individuals with Alzheimer's or mild cognitive impairment and individuals with no cognitive impairments (Adler et al., 2014). The results showed significantly lower amylin levels among subject with Alzheimer's and mild cognitive impairments compared to individuals with no cognitive deficits. With this correlation between amylin levels and cognitive function established, a follow-up study was conducted in order to investigate the effects of amylin administration on Alzheimer's pathology. A senescence-accelerated prone (SAMP8) mouse was selected as a model of Alzheimer's related dementia, because it displays multiple features known to occur early in the pathogenesis of Alzheimer's including cortical atrophy, amyloid beta alterations, tau phosphorylation and severe deficits of learning and memory. The SAMP8 mice were treated with either amylin or saline infusions for a total of 5 weeks. Due to the tendency of amylin to aggregate into brain plaques in its natural form, a soluble analog of amylin called pramlintide was used in the study.

The object recognition test was performed on the mice during the last week of the 5-week treatment period in order to assess the effects of amylin on cognitive function. The object recognition test is a behavioral assay that is based upon the natural tendency of mice to investigate a novel object instead of a familiar one, as well as their innate tendency to restart exploring when they are presented with a novel environment. The mice were placed in an open field box filled with different objects of various shapes and sizes. After a series of trials, during which the mice habituated to the configuration and properties of the different objects, some of the objects were replaced with new ones to evaluate novel object recognition. The pramlintide-treated SAMP8 mice spent a greater proportion of time exploring the novel objects as compared with the familiar objects, whereas the saline-treated SAMP8 mice did not differ in time spent with the novel and familiar objects. The behavior of the pramlintide-treated mice signified a marked improvement in their cognitive function.

Hippocampal tissue samples were harvested from the SAMP8 mice in order to assess the effect of amylin on oxidative stress, an important pathologic feature of Alzheimer's. The protein levels found in the pramlintide-treated SAMP8 mice indicated a decrease in the molecular markers associated with oxidative stress and neuro-inflammation. The pramlintide-treated SAMP8 mice had significantly decreased expression of the protein H0-1 in the hippocampus compared with saline-treated mice. HO-1 is a cellular stress protein that is activated during high oxidative stress and inflammatory states, and is also known to be increased in the cerebral cortex of Alzheimer's brains. The pramlintide-treated SAMP8 mice also had decreased levels of the lipid peroxidation adduct HNE and the enzyme COX-2. HNE is a protein that is known to be an early and abundant cellular stress marker in Alzheimer's brains, while C0X-2 is a classic marker of inflammation which is increased in Alzheimer's brains.

The pramlintide-treated SAMP8 mice expressed high amount of proteins associated with synaptic activity and dendritic growth. Amylin was found to increase expression of hippocampal synapsin I, a protein located in neuronal synaptic vesicles that is involved in synapse formation, neurotransmitter release, and learning and memory. A robust increase in CDK5 was also observed in the hippocampus of the pramlintide-treated SAMP8 mice. CDK5 is a kinase which plays an intimate role in synaptic plasticity, learning and memory in adult brains.

The overall conclusions of the study were that chronic infusion of amylin in SAMP8 mice was found to improve memory performance in object recognition tests, increase neural synaptic activity and decrease inflammatory markers in the hippocampus. Amylin treatment improved both the cognitive status and Alzheimer's pathology features of the SAMP8 mice.

Another study also assessed the results of amyloid treatment on behavioral impairment and brain amyloid pathology in mouse models of Alzheimer's disease (Zhu et al., 2015). The

study utilized 5XFAD Alzheimer mice which exhibit significant neuron loss. The 5XFAD mice were treated with intraperitoneal injections of either pramlintide or saline once daily for 10 weeks.

The 5XFAD mice were tested for improvements in cognitive functions by going through a Morris water maze. The Morris water maze test is used to determine hippocampal spatial memory deficits. The test consisted of placing the rodent in a circular tank filled with cloudy water, which is used to motivate the animal to escape the water by swimming to a hidden platform located in one quadrant of the pool. Over several days the rodent learnt to find the hidden platform by using spatial cues, such as posters or taped objects strategically placed on the walls outside of the water maze, in the testing room. The pramlintide treatment improved the performance of the mice in the Morris water maze test, reducing the time necessary for memory acquisition and retention during maze training as compared to the saline-treated control group. The improved performance of the pramlintide-treated groups over the saline-treated groups in the Morris water maze test demonstrated that peripheral treatments with pramlintide improves learning and memory in the 5XFAD mice.

Pramlintide treatment was shown to have an effect on amyloid beta levels, one of the prime pathological hallmarks of Alzheimer's. Immunoassays of brain tissue from the 5XFAD mice revealed that pramlintide-treated 5XFAD mice experience a reduction in both size and intensity of amyloid beta plaques in the cortex, hippocampus and thalamus and had decreased numbers of amyloid beta plaques in all areas of the brain with the exception of the hippocampus. Furthermore, comparison of amyloid beta serum levels from before and after the intraperitoneal pramlintide infusion revealed a significant increase in serum amyloid beta after the infusion. These results indicate that pramlintide enhances the removal of amyloid beta from the brain and its transfer into the blood.

The successful outcomes of these separate studies assessing the effect of pramlintide treatment on different Alzheimer's mouse models indicate that amylin has potential to become a promising new avenue for the treatment of Alzheimer's. Amylin was shown to improve cognitive function, reduce oxidative stress markers, increase synaptic activity and enhance clearance of amyloid beta from the brain.

Leptin

Leptin is a chemical produced by fat tissues which activates the central nervous system to regulate food consumption and energy expenditure (Oomura et al., 2006). Although leptin has traditionally been studied in the context of obesity, recent studies have examined its neurological effects. Leptin receptors are highly expressed in areas of the brain that are involved in learning and memory, such as the hippocampus (Li, X-L et al., 2002).

A study was designed in order to assess leptin's role in the regulation of hippocampal functions and the control of learning and memory processes (Li, X-L et al., 2002). The study focused on leptin receptor-deficient mice. The behavioral and molecular data obtained from the leptin receptor-deficient mice was compared against a positive leptin receptor control group. Both groups of mice were put through the Morris water maze task. The leptin receptor-deficient mice swam greater distance than their positive controls before they found and climbed onto the hidden platform. When the platform was removed, the leptin receptor-deficient mice crossed the original platform location fewer times than the positive control group. The hippocampus was removed from the leptin receptor-deficient rodents and the control group. Electrophysiological analysis of the hippocampal tissue of the leptin receptor-deficient mice showed impairments of long term potentiation (LTP) and long term depression (LTD). The decreased cognitive behavior and impaired LTP and LTD processing observed in the leptin receptor-deficient mice support the conclusion that leptin enhances LTP and regulates mechanism involved in both learning and memory

Leptin also appears to regulate a number of defining features of Alzheimer's disease. AMP- dependent kinase (AMPK) is known to regulate glycogen synthase kinase-3B (GSK-3B), a kinase which is crucial in the regulation of tau phosphorylation (Nikolaos et al., 2009). It has been shown that leptin directly activates AMPK and thereby possesses the ability to modulate tau phosphorylation (Yu et al., 2004). Leptin also facilitates the uptake of amyloid beta complexes via its regulation of the lipoprotein receptor-like protein (Fewlass et al., 2004). Thus, leptin's activity directly affects the regulation of amyloid beta uptake and tau phosphorylation, two of the impaired pathophysiological features of Alzheimer's disease.

One study assessed the effects of leptin treatment on CRND8 Alzheimer's mouse models (Greco et al., 2010). The CRND8 mice received daily treatments of leptin for a total of 8 weeks with a control group receiving saline infusions. Leptin-treated mice spent statistically more time with the novel object compared to the saline-treated control group during the object recognition test, indicating an improvement in working memory performance after leptin treatment. Analysis of brain tissue from the CRND8 mice revealed that the leptin-treated group had reduced amyloid beta levels in both brain and serum. Staining of the brain tissue for amyloid fibers showed a significant decrease in amyloid burden in the hippocampus of the leptin

treated CRND8 mice, which was associated with a decrease in the average size of amyloid plaques. There was no significant increase in the levels of the inflammatory molecule C-reactive protein, tumor necrosis factor or cortisol in the plasma of the leptin-treated group compared to the saline-treated group, indicating that leptin does not induce an inflammatory reaction. The results of the study fully support the ability of leptin to ameliorate Alzheimer's like pathological pathways, strengthening leptin's potential of becoming a novel therapeutic treatment for Alzheimer's disease.

Synergistic Effects of Amylin and Leptin

Leptin and amylin activate overlapping signaling cascades and ultimately converge on the insulin signaling pathway by activating AKT and increasing insulin sensitivity (Yarchoan and Arnold, 2014). Recent studies have indicated that leptin and amylin signaling appear to have synergistic properties.

One study profiled hypothalamic neurons in order to determine the effects of amylin and leptin on hypothalamic activity (Li, Z et al., 2015). It was discovered that hypothalamic expression of lapp, a precursor to amylin, was markedly decreased in mice with mutations in the gene regulating the production of amylin, but was normalized after infusions of leptin. The decrease of amylin expression in mice that had mutated leptin genes showed that hypothalamic amylin is a neuropeptide that is leptin regulated. Additionally, AC187, an amylin antagonist, was found to blunt the activity of leptin and decreased its effects on neurons in the hypothalamus. The presence of the amylin antagonist significantly inhibited the effects of leptin on both leptin depolarizing and hyperpolarizing neurons. The ability of an amylin antagonist to blunt the response of leptin suggests that amylin can modulate leptin's effects. Leptin and amylin were also found to have synergistic effects on hypothalamic neurons. Patch clamp recordings demonstrated that the presence of either leptin or amylin elicited similar excitatory and inhibitory effects on hypothalamic neurons. Leptin excited 65% and inhibited 35% of the neurons, while amylin excited 62% and inhibited 38%. The significant correlation between the effects of individual neurons exposed to both treatments indicates that amylin depolarizes the same neurons that are depolarized by leptin and hyperpolarizes the same neurons that are hyperpolarized by leptin. This suggests that the response of the neuron would be amplified upon simultaneous presentation of amylin and leptin.

The synergistic potential of these two treatments has been explored with regards to treating obesity. Stand-alone obesity treatments have proven unsuccessful because diet-induced obese (DIO) rats and obese humans are only minimally responsive to even high pharmacological doses. Nonetheless, amylin possesses the ability to heighten leptin's effects. For this reason, one study found that doses of exogenous leptin that was highly effective in lean rats had minimal effects on weight or food intake in DIO rats. However, the results of the study showed that administration of amylin together with leptin resulted in a synergistic, fat-specific reduction in body weight in two independent experiments (Roth et al., 2008).

In light of this discovery, a clinical trial was designed in order to evaluate the weight-lowering effect of combined amylin/leptin (using pramlintide/mertreleptin) treatment in human obesity. A 24-week, randomized, double-blind, study was conducted in obese or overweight subjects. Subjects receiving pramlintide/metreleptin lost almost 13% of their initial body weight over 24 weeks, compared with only ~8% in subjects receiving either pramlintide or metreleptin. Towards the end of the study, weight loss plateaued in subjects treated with monotherapy, but not in subjects treated with the combination. The overall results of the study showed that weight loss caused by the combination of leptin and amylin in humans was greater than the additive weight loss of each drug used alone (Ravussin et al., 2009).

Thus far, there have been no studies to examine the efficacy of combination therapy of leptin and amylin to treat Alzheimer's. The successful results observed in weight loss clinical trials suggest that even greater improvements in memory in Alzheimer's patients may be possible by using amylin and leptin as a combined therapy.

Conclusion

The discovery of insulin resistance in Alzheimer's has given way to a growing interest in restoring insulin signaling in Alzheimer's with therapeutic agents originally developed for the treatment of Type 2 Diabetes. Intranasal insulin, amylin and leptin are examples of hormones typically associated with obesity and diabetes which have shown promise for treating Alzheimer's disease. An advantage of using amylin and leptin as medications for Alzheimer's is that unlike with insulin, treatment using these hormones pose no risk of inducing hyperinsulinemia, as they activate the products of the insulin signaling pathway without interfering with the concentration of insulin in the body. Furthermore, weight loss studies have discovered that amylin and leptin are synergistic substances which produce significantly enhanced results when used in combination. This unique synergy suggests that achieving even greater improvements in memory may be possible by using amylin and leptin as a combined therapy for Alzheimer's. Further research will need to take place before the proposed combined therapy can be tested in a clinical trial and ultimately be distributed pharmaceutically.

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The Impacts of Childhood Obesity on Adult Health and Quality of Life

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Abstract
Obesity has emerged as one of the most preeminent concerns of the modern era. Thirty six percent of our country's citizens are classified as obese and six percent qualify as extremely obese. Developing obesity as an adult is fairly common, but countless studies have shown a direct correlation between childhood obesity and remaining severely overweight as an adult. Aside from the physical and aesthetic discomfort of maintaining superfluous girth, the health hazards threatening the obese population are extremely discomfiting. The enormity of the issue requires extensive study so that society can educate themselves of the dangers and how to prevent them. The purpose of this paper is to explore the ramifications of childhood obesity on adult quality of life; given the probability that overweight youth will remain that way, it is vital to determine the groups at risk for obesity and what diseases they're likely to be at risk for. The studies discussed support the theory that obese children will usually remain that way and that they are at even higher risk for diseases like diabetes and heart disease than their obese peers who had not suffered from childhood obesity.

Part I-Introduction:

The last three decades have witnessed an enormous increase in childhood obesity in the United States. It is estimated that at least 17% percent of children are currently overweight according to the Centers for Disease Control and Prevention (CDC), and certain demographics like Hispanic and Black Americans display more alarming percentages. As more children become classified as overweight, those already at risk for extreme obesity are becoming even heavier. This becomes a grave concern because an adolescent develops an acute probability of becoming an obese adult. Consequently, childhood obesity has evolved into the most widespread predominant nutritional disorder affecting American children that pediatricians address (Childhood Overwieght, 2014).

Contributors to Childhood Obesity

The factors that contribute to this serious health concern are numerous. The most obvious ones are food choices consumed in an individual's diet, and average physical vs. sedentary activity. Less obvious but still pertinent are hereditary and genetic factors. There are numerous genetic alterations, or single gene mutations that are responsible for weight gain, although this is rarer (Childhood Overwieght, 2014). Parental obesity is a very significant indicator of a child's potential for being overweight. Specifically, parental BMI preceding and during pregnancy is a dominant early-life risk factor influencing BMI of offspring in adulthood. BMI is an acronym for body mass index which is a figure that relates weight to height and is obtained by dividing a person's weight in kilograms by his or her height in meters squared (Definition of Body mass index, 2016).

In the Australian pregnancy cohort study, offspring of mothers who had not received a college education, smoked and gained excessive weight during pregnancy or had high BMIs preceding pregnancy, had a higher chance of being obese at age 22. Paternal obesity, bottle fed babies and those who had a high birth weight had an increased risk of childhood but not necessarily adult obesity (Rath et. al. 2016).

Physical Consequences of Childhood Obesity

Adolescents who suffer from extreme overweight issues are prone to a host of severe medical issues at every stage of life, amongst them problems that normally afflict only the senior population. A well-documented study indicates that the lifetime risk of being diagnosed with type 2 diabetes (a disease brought on by excess weight) is estimated at 30% for boys and 40% for girls. Other publicized studies of the health issues directly or indirectly caused by obesity include cardiovascular disease, hypertension, high cholesterol, and in some rare but disturbing incidents, heart attacks in children as young as 5 (Bagchi, 2011).

Children who suffer from these contributing factors to heart disease may be suffering from Metabolic Syndrome (MS), which refers to a group of risk factors that increases the danger of developing heart disease and other health problems, such as diabetes and stroke. (What Is Metabolic Syndrome?, 2016).

In a study to determine MS prevalence, 201 obese subjects aged 6 to 18 were studied. The subjects in the study were diagnosed with MS if they had a BMI above the 97th percentile and met two or more of the following conditions: triglyceride level ≥ 1.7 mmol/L, HDL ≤ 1.03 mmol/L, fasting glucose ≥ 5.6 mmol/L, and arterial hypertension (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg). Of the 201 children and adolescents, 110 (54.7%) subjects were moderately obese and 45.3% were categorized as severely obese. The frequency of MS was 24.5% in the group of moderately obese and 37.4% in severely obese subjects. The majority of children and adolescents in the study had one or two components of MS. This discovery highlights the significance of recognizing degrees of obesity in children and adolescents, which could theoretically influence different rates of displaying cardiovascular risk factors (Šimunović et. al. 2016).

The obese pediatric population is also prone to developing nonalcoholic fatty liver disease (NAFLD). In a Japanese study conducted on NAFLD, researchers determined that more than 10% of all obese children had at least modest increases in serum transaminases, which indicates liver damage, even if they hadn't

developed full NAFLD. Other consequences of obesity include cholelithiasis, pseudotumor cerebri, obstructive sleep apnea, polycystic ovary syndrome, and orthopedic conditions such as slipped capital femoral epiphysis (Allcock., 2009).

Psychological Impact

Aside from the many alarming medical conditions these children grapple with, the detrimental impact their circumstance can have on their emotional wellbeing can be devastating. Obese and overweight children often develop depressive symptoms that can lead to negative body image and put them at risk for developing an eating disorder. Consequently, these children suffer from low self-esteem, which can instigate behavior issues that may develop into learning difficulties (Childhood Overwieght, 2014). A well detailed study on the psychological effects of childhood obesity found that children aged 5 to adolescents aged 18 shockingly compared their quality of life to individuals undergoing chemotherapy treatment for cancer (Bagchi, 2011).

A study involving young school age girls was conducted to determine how much behavior is impacted by weight. The study observed behavioral problems in 17%, 27%, and 2% of obese, overweight, and normal weight children, respectively. Cultural differences between varying countries didn't impact the correlation between the two factors and similarities were observed in most of the emotional-behavioral problems related to overweight and obesity. Internal emotional baggage, which includes anxiety, depression and withdrawal were seen in 11%, 15%, and 2% respectively, and external indicators of emotional disturbance, including aggression and delinquent behaviors, were observed in 8%, 17%, and 2% in obese, overweight, and normal weight children, respectively. (Seyedamini, et. al. 2012)

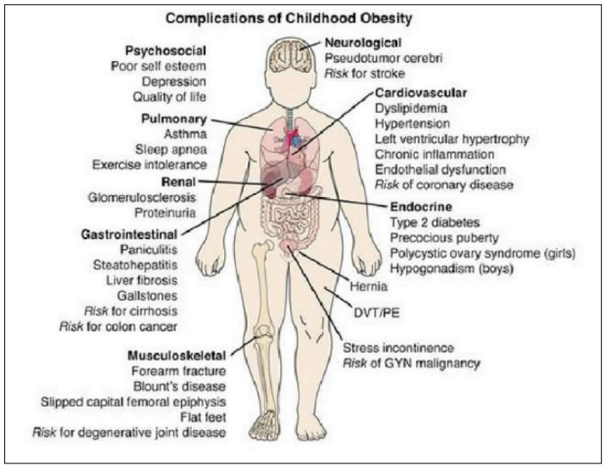

Figure 1 A diagram of a child's obese body with labels on the parts of the body that can be affected by obesity (Williams, 2010).

The data we have concerning childhood obesity and its correlation to significant impact on the physical and emotional health of the child is concerning enough. But the consequences on health later in life also requires study. Thus, the essential question in this field is: What effects will adolescent obesity have on the health of the child when s/he becomes an adult?

This paper will examine the various health issues that can surface for individual who continue to suffer from extreme overweight into adulthood, in addition to proposing some solutions for those who fall into that category.

Methods

Information was obtained online with access to online publications through the Touro college library. Additional references were obtained through Pubmed.

Part 2- Results and Discussion: Correlation Between Childhood Obesity and Adult Obesity

Before determining what the medical impacts on adult health are due to childhood obesity,we need to answer a fundamental question: Do obese children become obese adults? According to the response of test subjects, roughly twice as many individuals who reported that they were "considered a fat child" were more than 155% larger than what their ideal body weight should have been as young adult women, compared with women who did not report that they were "considered a fat child" (Rimm, 1976). Corpulence in infants leads to a twofold increase for risk of subsequent obesity. Another study concluded that the odds ratio for the persistence of obesity rises with age among obese children; endurance of obesity for overweight children hovered at 50%. Considering that this study was conducted before the enormous surge in childhood obesity in recent years, the current figure would be even higher (Allcock et. al., 2009).

A study was conducted where 1,355 participants from the Australian Pregnancy Cohort were analysed. This represented an Australian birth group born between 1989 and 1991. There were 12 periodic intervals where mothers and children were surveyed starting from early pregnancy: birth, 1, 2, 3, 5, 8, 10, 14, 17, 18, 20 and 22 years. The data collected provided researchers with an opportunity to track BMI patterns from birth to maturity and to examine the significance of early-life contributing factors to obesity.

Factors that were investigated as possible causes of being extremely overweight were: sex, most advanced level of education of the mother (paternal education level not available), maternal and paternal pre-pregnancy BMI (based on recalled weight recorded at 18 weeks of pregnancy), maternal smoking during

pregnancy, maternal anemia and diabetes during pregnancy (pre-existing or gestational), and excessive pregnancy weight gain (change in weight from pre-pregnancy to 18 weeks and 34 weeks' gestation). These factors all represent pre-birth conditions. Post-partum, other factors of influence were observed to be cesarean delivery, early birth, birth-weight, first-year growth and extent of breast-feeding.

BMI of the participants was recorded; to eliminate any gender discrepancy the BMI was standardized using z scores and standard deviations. A BMI higher than the 85th percentile was considered overweight and obesity was declared above the 95th percentile. In adulthood, these would correlate to BMIs of 25 and 30 respectively.

The results indicated that maternal and paternal BMI produced the strongest effect on offspring BMI, particularly in adolescence and early adulthood. Compared to children whose parents were not overweight, children at ages 14 and 22 with overweight mothers had more than 4 times the risk of developing obesity. This paled in comparison to children of obese mothers which predicted a 7to10-fold increase of risk. Paternal overweight almost tripled obesity risk at age 14 although it was determined to be non-impacting at age 22. But paternal obesity resulted in an almost fourfold increased risk of offspring obesity at both ages (Rath, 2016).

In an attempt to clarify the correlation between childhood and adult obesity, statistics were assembled from four longitudinal studies conducted between 1929 and 1960. The studies compared the BMI at the target adult age of 35 to the BMI between 1 and 18 years. BMI at age 13 usually predicted the BMI at 35 and the BMI at 18 was usually extremely accurate in predicting adult size. One study tracked the BMI of infants until the age of 21. At the study's completion, 41% of the individuals that were lean at one year remained in the lean category and 41% of the fat infants remained in the fat category. Further studies have projected that up to 81% of overweight (BMI greater than 85th percentile) adolescents will become obese young adults. (Whitaker, 1997)

Researchers have also had to determine which BMI should be the cutoff point that ascertains obesity. They established that the likelihood that obesity will continue in 50% of adolescents identified by a BMI ≥95th percentile, or by a weight-for-height of 130%, which is slightly larger than a BMI ≥95th percentile, suggests that a BMI ≥95th for children of the same age and sex signifies a realistic cutoff point to identify lasting obesity. After establishing these percentage guidelines, researchers then had to establish the number adolescents that fall into these categories. The CDC has determined contemporary data that demonstrates that 31.9% of youth aged 2-19 are at or above the 85th percentile, 16.3% are at or above the 95th percentile and a shocking 11.3% are at or above the 97th percentile of the 2000 BMI-for-age growth charts. This indicates that approximately one in three individuals between the age of 2 and 19 years is either overweight or obese (Allcock., 2009).

Health Implications for Obese Adults

Now that we have determined the immense likelihood that obese children will morph into obese adults, we need to examine the health consequences this correlation presents. Individuals who suffer from childhood obesity are at risk for higher rates of developing disease as an adult as well as dying earlier. This can be referred to as the impacts on adult morbidity and mortality, which is defined in the following way: Morbidity describes the unhealthy state of an individual while mortality occurrence of death in a population (Morbidity vs. Mortality, n.d.).

A 1988 follow up on the Third Harvard Growth Study of the early 1930s that tracked school-age youth into adulthood provided some insight into the effects on adult health and quality of life. Overweight participants were determined to be adolescents who had more than two measurements of their body mass index (BMI) above the 75th percentile during high school. Subjects with a BMI between the 25th and 50th percentile throughout high school were classified as slim. The follow-up data was collected when the subjects were 55 years of age, and the results were modified to represent smoking status and reported weight. There was an extremely high correlation between disease and their excess childhood weight. Both males and females had increased rates of the classic diseases that afflict the obese population: diabetes, coronary heart disease, atherosclerosis, hip fracture and gout. The risk of these diseases was only slightly diminished after adjustment for smoking and adult weight. Only the risk of diabetes became insignificant after adjustment for lower adult weight; type 2 diabetes is known to be directly correlated to high weight (Dietz, 1998).

A Dutch study similarly sought to observe the impact of a higher BMI at age 18 on premature adult mortality from all causes. A group of over 78,000 Dutch men at the age of 18 were studied and tracked over a 32-year period. They found a higher incidence of all-cause deaths in those individuals with a BMI >25 (classified as overweight) compared to individuals with a BMI between 18 and 25 (Hoffmans, 1988).

Cardiovascular Disease and Risk Factors

Of all the diseases mentioned, the most severe are arguably cardiovascular. How does childhood obesity impact adult heart health, or lack thereof? With rising evidence to help conclude that childhood obesity is linked to adult obesity, the medical world has been grappling with the question as to whether obese

adults' risk of cardiovascular disease (CVD) is increased by their pediatric obesity or just similar to weight-matched peers.

The precise connection between obesity in youth and adult development of cardiovascular disease was researched in the Princeton Follow-up Study. As expected, the group that presented symptoms of MS in childhood due to obesity had a dramatically increased risk of developing cardiovascular disease 25 years down the road, compared with the group that didn't suffer as children. In fact, MS in childhood was a stronger determinant of future cardiovascular disease in adulthood than was gender or even genetic history of cardiovascular disease. The youth in their initial study exhibited an adolescent incidence of MS of 4%. In the follow-up study, this had risen to 27.2% for adults. This study also demonstrated that risk of CVD increased by more than 24% for each 10 % increase of BMI (Allcock, 2009).

Researchers also wanted to establish the connection between weight change or maintenance from youth to adulthood and the subsequent development of MS.

From 1993-4, a population study examining participants born between 1947-57 was implemented in Finland. Records of the 7-year-old height and weight were obtained for 439 subjects. Obesity was delineated as the top third BMI measure and MS was diagnosed based on observations in the following areas: a systolic blood pressure ≥140 mm Hg and a diastolic blood pressure ≥90 mm Hg. If a patient was receiving treatment of antihypertensive drugs they were considered to have high blood pressure as well. Other factors were: dyslipidaemia (hypertriglyceridaemia ≥1.70 mmol/l, low HDL cholesterol levels <1.00 mmol/l (<1.20 mmol/l in women), insulin resistance (abnormal glucose metabolism according to the World Health Organization's criteria or hyperinsulinaemia (≥78 pmol/l), or both).

The number breakdown amongst the 439 subjects was as follows: 75 were obese as both adults and children, while 219 weren't obese for either age. Of these, 71 were obese adults who hadn't been fat as children, and another 74 were overweight in their youth but not as adults. Eight per cent of men and 5% of women displayed symptoms of MS, which amounted to 30 individuals in total. Of those people, 21% were obese adults and 21 had also suffered from childhood obesity. The study found that risk of MS was 2.9% for adults who had been obese as children but this figure increased to 26.7% for people who continued to obesity into adulthood. Interestingly there was a zero incidence of MS in the 74 people who had formerly been overweight but had since slimmed down.

The study concluded that ultimately childhood obesity augmented the dangers for developing MS as an adult and that obese youth who grew to be obese adults had a particularly high risk of MS. Incredibly, the risk was lower in obese adults who had not been that way as children. This reinforces the theory that obesity determined prior to adulthood is more detrimental than late onset obesity. The probable explanation for this is that unceasing obesity serves as a "generator" for protracted insulin resistance, which is directly correlated to and causes hypertension and metabolic irregularities. The study results indicate that prevention of adult obesity and consequent MS and CVD risk is dependent on the earliest possible intervention for obese children to ensure they lose the weight (Vanhala, 1998).

One of the indicators of CV. is carotid artery intima-media thickness (IMT) and is recognized as an important predictive measure of clinical coronary atherosclerosis health issues in middle-aged and elderly populations. Carotid artery IMT measures the thickness of the inner two layers of the carotid artery, the intima and media. A thickening of both is a measure used to diagnose the extent of carotid atherosclerotic vascular disease (Carotid Intima-Media Thickness Test, n.d.).

From September 1973 to December 1996, a study that investigated the effect of childhood adiposity on adult heart health examined a cohort of 486 participants from a community in Bogalusa, Louisiana. Surveys were conducted approximately every 3 to 4 years; 7 surveys examined children from the ages of 4 to 17 years while 5 surveys followed up with young adults aged 18 to 38 years, who had participated as children and remained available. The premise of the study (observing children into adulthood over a 20-year span) enabled the researchers to measure the collective and snowballing effect of risk factors since childhood. The purpose of the study was to observe the link between carotid IMT in young adults and traditional cardiovascular risk factors measured since childhood.

Participants experienced the following procedure for each examination: Height and weight were obtained within a .1% range of error after the subject fasted for half a day. These measures were used to calculate BMI. Next, duplicate blood pressure measurements were taken. Systolic and diastolic blood pressure levels were evaluated 6 times and these levels were averaged for the final observation.

The results found strong correlation coefficients between CVD risk factors measured since childhood and the carotid IMT in young adults: Overweight adolescents with high levels of LDL cholesterol ("bad" cholesterol) in childhood, high BMI and systolic blood pressure were interrelated with carotid IMT in young adults, with LDL-C level showing the highest correlation. As mentioned previously, this is concerning as high carotid IMT levels are strong indicators of developing cardiovascular disease. (Li S 2003)

Prostate Cancer and Obesity

Prostate cancer has also been linked to obesity. When the prolonged nature of this disease is considered, it can be assumed that adolescent health may be an early life contributor. A number of studies of the correlation between prostate cancer and early life obesity. It is important to note that the results of the studies provided conflicting data: some observed a direct link between childhood BMI and prostate cancer risk, while others actually determined that increased BMI served as a protective measure against the disease.

A contemporary study that tracked 950,000 men for a 20-year span was conducted in Norway. The study determined that a BMI larger than 30 amplified the risk of prostate cancer by only 9%. Conversely, the 50 to 59 age group of obese men had a 58% increased risk of prostate cancer but no younger age group presented a substantial risk. Another study that incorporated data from the Health Professionals Follow-up Study by Giovannucci and colleagues, discovered that an increased BMI was actually correlated to a decreased risk of prostate cancer among men younger than 60 years or those with a family history of the disease (Freedland and Aronson, 2004).

Psychological Ramifications of Childhood Obesity into Adulthood

Aside from the obvious physical implications obesity presents, the Harvard Growth study also determined that additional psychological repercussions resulted when adult morbidity was linked to childhood obesity. In a large sample studied during the National Longitudinal Survey of Youth, surveyors revealed that obesity present in adolescent females had grave social consequences several years later as an adult. Amongst them were: low marriage rates, decreased years of education, inferior net incomes and compounded poverty levels. The prevalence of these results, (after adjustment for the income and educational levels of the individual's family as well as self-esteem) indicated that female obesity actually triggered these socioeconomic links, as opposed to being a result of these socioeconomic associations. This further demonstrates the detrimental impacts of adolescent obesity on adult quality of life. (Dietz, 1998)

Part 3: Future- Preventions and Treatment Youth Intervention Studies

Looking forward, it is incumbent on us as a society to prevent and treat obesity given all the severe and often fatal maladies obesity causes. So far, exploration of obesity prevention was conducted in only a limited number of studies. This may be for a host of reasons including a dearth of funding for public health and obesity investigation; insufficient prevention tactics, and overall inadequate training in medical education programs. The prevention tactics targeting obesity on a small community-wide scale that have been used are the following: Targeting a community wide population with the goal of reducing the average BMI collectively, which is known as an intervention strategy. If a strategy narrows the scope to target only individuals already at high risk (offspring of obese parents) it is a selective prevention. This kind of program seeks to educate and imbue these people with the necessary skills to avoid weight gain. Finally, a targeted prevention tactic assists those who are already overweight or obese in their attempt to lose weight. One example of how this was implemented was in the North Carelia Project which incorporated media education, as well as programs specifically designed for schools and work places. The program saw the most success in a ten year follow up when children were treated in conjunction with their parents. Weight changes and decreased incidence of obesity proved the efficacy of the family based and lifestyle mediations.

Researchers on the subject believe that primary intervention for youth is better than attempting to treat already obese adults. There are established periods that have been identified as crucial to preserving healthy body weight: the prenatal stage, the period between 5 and 7 years of age (which is referred to as the adiposity rebound) and the teenage years. Although a large percentage of individuals may only experience weight gain as adults, there is a substantial correlation between childhood and adult BMI. Childhood obesity persevering into adulthood appears to rise linearly as a child get older. Precursors of adult diseases such as hypertension are increasingly affecting obese children. As demonstrated earlier, childhood obesity is a strong predictor of adult morbidity and it has therefore been suggested that an effective treatment of adult obesity may be youth intervention. Based on this assumption, researchers developed the KOPS (Kiel Obesity Prevention Study) which inaugurated in 1996. A cohort of 5 to 7 year old children was gathered in a the city of Kiel, Germany and the study was supervised with cooperation from school physicians and teachers, in addition to the formation of a new sports program for heavy children. Basic information gathered at the beginning of the study included: evaluation of the nutritional state and dietary habits of the child, review of lifestyle (active vs. sedentary) social status, physical fitness compared to a subgroup of children, muscle strength, and preexisting health factors that increased risk, such as blood pressure, glucose levels and cholesterol. The cohort (which amounted to 25,338 children in total) had their BMIs recorded in comparison to the norms of the total group of 5-7 year olds born in 1998. Overweight and obese BMIs were designated based on the following criterion: triceps skinfold thickness (TSF) as a parameter of fat mass. The 90th TSF percentile was used as the value to determine obesity. The study was designed to conduct a follow up session with the participants every 4 and 8 years. The study was implemented by introducing a nutritional

awareness and health promotion program for children and their parents in three intervention schools every year. Corresponding data from demographically similar schools were designated as the control groups. This reversed every alternate year. Family assistance and a structured sports program were offered to families with overweight or obese children, as well as families with normal-weight children with obese parents.

The premise of the study was that preventing obesity is dependent on healthy lifestyle. Lifestyle changes can only be implemented by augmenting knowledge and self-awareness as well as harnessing self-esteem and personal independence. The rigorous intervention methods included a panoply of directives, amongst them: eat fruit and vegetables daily, reduce the intake of high fat foods, keep active at least 1 hour a day, and decrease TV consumption to less than 1 hour a day. These messages were delivered to pre-school age children within their first year. Teachers working in conjunction with skilled nutritionists offered 8 hour courses on nutrition for the students as well as parents, who were asked to attend school meetings to address the issue. Aside from the programs offered in school, families with obese children or parents were provided with counseling and support programs for the family as a whole. This consisted of home visits arranged by a nutritionist who visited on between 3 to 5 times in a span of 3 months to assist with shopping, cooking and 'resetting the family table'. The counseling instructed parents to screen food ingestion and physical activity. A half year program of structured sports biweekly was also offered to overweight youth. Assessment of the results were collected with the intention of determining the following outcomes: primarily, a change in BMI and second, change in health-related comorbidity, or the presence of diseases caused by obesity. The differences were examined in both the intervention and the control schools.

In the first 4 years of the study, school interventions were directed at 414 children, their parents and teachers. 92 out of 368 eligible families partook in the family intervention program and 25% of the families finished the program. The short-term effects were studied for all children within 3 months after the end of the interventions. Due to the interventions, 60% of the children exhibited adequate nutrition recognition (as opposed to the original 48%), 65% of children related that their physical activity had increased (while time spent in TV watching had diminished) and 28% of them had joined a sports group. Families as whole had a 50% increase in their produce consumption and low fat food was also consumed more frequently, increasing from 20% to 50%. The nutritional state of children in the intervention schools was reevaluated after 1 years' time. Compared to the nutritional states of children in control school, the intervention school group showed drastically improved results: the control groups had higher increases in TSF as well as percentage of fat mass. (Müller et al. 2001)

Another study was conducted to evaluate various methods of intervention for adolescents ranging from behavioral, which consisted of introducing simple intervention methods, to pharmacological treatments which consisted of a drug regimen, and finally, surgical interventions for the morbidly obese sector.

Behavioral interventions were incorporated in schools or specialty health care settings to target youth aged 5 to 18 years and introduced behavioral modifications similar to the Kiel Obesity Prevention study: education about healthy dietary habits and augmented physical activity. The data collected from the school interventions described a 0.4 to 2.07 difference in mean BMI change between those that were treated and the controls at 6 to 12 months, with a collective estimate of .82 lower BMI in those treated. This would mean a loss of 3 pounds for an 8-year-old boy or girl, (assuming growth of 2 inches or less), and a 4-pound loss for a 12-year old boy or girl. For a 16-year-old adolescent, this would mean a weight loss of 4.5-6 pounds, depending on gender. Specialty health care setting proved to be much more effective, displaying a 1.9 to 3.3 BMI difference, compared to control groups, for 6–12 months post-treatment. This would mean a 12-13-pound weight loss for an 8 year old (again, assuming 2 inches of growth), and a 16.6 to 17.75 pound weight loss for a 12 year old. 16 year olds enrolled in the program could experience up to a 23-pound weight loss (Whitlock EP et al. 2008).

It is important to acknowledge that the trial results would not necessarily translate well to helping individuals in a real world setting. The studies utilized media advertisements for enrollment; it follows that those participants may have been more inspired to lose weight, and had a surplus of free time. Parental alarm, multiple failed attempts at weight loss or other such factors may have produced skewed results.

Pharmacological aides in addition to behavioral interventions have been investigated only in obese youth aged 12 to 18 years that meet adult criteria for class II obesity, which is a mean BMI of 35 to 40 the beginning of the trial. Drugs incorporated into the weight loss regimen such as sibutramine and orlistat were administered in conjunction with behavioral interventions for a 6-12 month span. Unfortunately, longer term impacts after treatment termination are not presented for any of the pharmacological trials (Whitlock EP et al. 2008).

A sizable trial consisting of 498 people tested 12 months of sibutramine in combination with a behavioral intervention plan. The control group received the same behavior modification plans but a placebo pill. Sibutramine is a medication that supports weight-loss by altering neurotransmitters within the brain. Nerves need to correspond with other nerves and neurotransmitters are what nerves produce to accomplish the

process. When neurotransmitters are sent out, they either attach to other nerves or they may experience reuptake, which is when the same nerve reabsorbs it. Sibutramine obstructs the reuptake of the neurotransmitters dopamine, norepinephrine, and serotonin. Preventing reuptake of neurotransmitters modifies the balance of neurotransmitters within the nerve cells and thus affects nerve function and interaction.

After 12 months, the subjects who took the 10-15 mg dosage of subatramine lowered their BMI by almost 3 units, which corresponds to a 14-pound weight loss. Astonishingly, the participants of the control group experienced a 4.2-pound weight gain. Interestingly, it seems the decrease in weight that occurs over a year with intense and successful behavior intervention rivals the achievements of the pharmacological interventions in the same time span, but more direct comparison would be necessary to substantiate the claim. (Whitlock, et al. 2008) Although this drug proved to be successful, it's important to note that it's no longer available in the U.S. for fear of heart attack or stroke (Eni Williams, 2015).

Conclusion

Childhood obesity is increasing with alarming frequency and can be attributed to a number of factors including diet, health education, and parental BMI. The impacts can be devastating; obese children battle with deadly diseases that can severely compromise their quality of life. This issue only gets amplified if obesity persists into adulthood. Numerous studies have demonstrated the adult health ramifications caused by childhood obesity, including diabetes, metabolic syndrome and cardiovascular disease. Overall mortality for adults also increases when they have a BMI above 25 (overweight range). Although the present seems bleak, various studies have investigated prevention and treatment for obesity, amongst them community wide behavior interventions that have been very successful. If obesity is effectively conquered in childhood, the individual's adult health and quality of life can be immeasurably improved.

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Are Contact Lenses an Effective Vehicle for Ocular-Disease Drug Delivery?

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Abstract

Due to numerous drawbacks with current modes of treatment for various ocular diseases, researchers are synthesizing drug dispensing contact lenses. The lenses will contribute to greater bioavailability of the drug, the minimization of negative side effects, and increased patient compliance. As treatment for glaucoma, in vivo studies have been conducted with latanoprost, timolol maleate, and brimonidine tartrate-eluting lenses, and have succeeded in reducing intraocular pressure to desired values (Ciolino et al., 2016), (Schultz and Mint, 2002). As treatment for fungal keratitis, in vitro studies prove that econazole and natamycin-eluting contact lenses have been successful in killing 100% of fungi for sustained periods of time (Ciolino et al., 2011), (Phan et al., 2013). Finally, for allergic conjunctivitis, contact lenses containing nanoparticles of prednisolone have been synthesized and demonstrate effective drug-releasing capabilities (ElShaer et al., 2016).

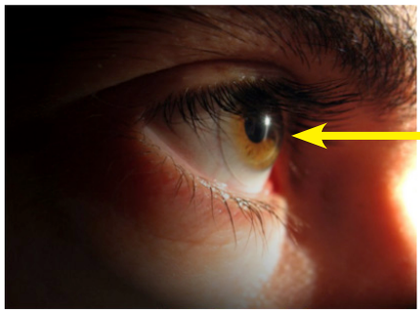
Introduction

Current methods of treatment for various ocular ailments include both oral medications and topical eye drops. There are significant downsides to both. Orals are often not the first line of treatment both because, they take a circuitous route to the eye and cause many more negative systemic side effects (Kim, et al., 2014). Next, in the case of eye drops, there are multiple barriers to overcome. First, much of the dispensed eye drop is inhibited by pre-corneal factors which include nasolacrimal drainage, tearing, and blinking. These factors significantly lower the bioavailability of the medication. Research indicates that only a fraction of the precious medication, a mere one percent to seven percent, reaches its required destination, thereby reducing the drug's effectiveness (Schultz and Mint, 2002). Furthermore, the drops are often administered by the patient, and sometimes are required multiple times a day. This commonly leasds to low patient compliance, and doses are frequently forgotten or skipped purposely (Ciolino et al., 2011). Given the above, there exists an impetus to develop alternate methods of delivering ocular medications, thus enabling effective treatment. Researchers are currently working on developing a contact lens that will also dispense nanoparticles of medication directly into the eye while correcting refractive error. In patients who don't have refractive error, the contact lenses can simply be worn for the purpose of delivering the needed medication into their eyes. The use of contact lenses for ocular drug delivery can solve many of the issues associated with eye drops. First, the space created by the lens with the cornea has limited tear mixing, and potentially a greater amount of contact time between the drug and the cornea.

This causes greater bioavailability. Additionally, there is an added benefit of eliminating the need for multiple doses a day, which will increase the amount of patient compliance. Under ideal kinetics, the drug will release in a time dependent manner, extending the therapeutic effects of one dose (Phan et al., 2014). Research with a drug dispensing contact lens (DDCL), is currently underway for a number of ocular conditions. In this work specifically, a DDCL for the diseases of glaucoma, fungal keratitis, and hay fever are discussed.

Glaucoma

Glaucoma, a group of conditions that damages the eye's optic nerve, usually results from increased intraocular pressure (IOP) which can result in vision loss and blindness. The two main forms are open-angle glaucoma and angle-closure glaucoma. Both forms, involve clogging of the eye's drainage canals, leading to elevated ocular pressures and subsequent nerve damage. In open-angle glaucoma this leads to a gradual increase in IOP because, the angle between the iris and cornea is wide and open. In angle-closure glaucoma there is a sudden



increase in IOP because, the angle between the iris and cornea is either very narrow or closed. Current methods of treatment include surgery, oral medications, and eye drops, depending on the classification and extent of the disease. When surgery is warranted however, it usually does not resolve the increased IOP completely and generally a regimen of eye drops are prescribed as well post operatively. Additionally, surgery can cause negative side effects including, cataract formation, inflammation, ocular infections, corneal issues, and low IOP. Negative side effects caused by oral medications can include irritation, stinging, redness, blurred vision, itchiness, low blood pressure, fatigue, shortness of breath, headaches, dry mouth, frequent urination, upset stomach, and memory problems, depending on the class of drug prescribed. In general, topical eye drops are the first line of treatment. However, due to the issues posited above, a contact lens that can dispense anti-glaucoma drugs is currently being developed.

Research for Anti-Glaucoma Drug Dispensing Contact Lens

Joseph B. Ciolino, MD, at the Massachusetts Eye and Ear, conducted an in vivo study on the effectiveness of a Latanoprost-dispensing contact lens for female monkeys with induced glaucoma. Latanoprost is currently on the market as a topical anti-glaucoma eye drop. It belongs to a class of anti-glaucoma medications knows as prostaglandin analogues, which work to lower the IOP by increasing uveoscleral flow (although more recently research suggests that it may occur through a trabecular pathway) and it is prescribed for cases of open-angle glaucoma (Lindén and Alm, 1999, Winkler and Fautsch, 2013). In Ciolino's research, a thin latanoprost-polymer film was introduced into a methafilcon hydrogel contact lens. Both a low-dose contact lens (CL), and a high-dose CL were synthesized. The intraocular pressure of the glaucomatous monkeys was monitored after a period of the following cases:

1. Treatment with Latanoprost eye drops
2. Treatment with CL-low
3. Treatment with CL-high
4. No treatment

The results demonstrated that the latanoprost eye drops succeeded in reducing IOP approximately 5 mmHg, the CL-lo by about 6.5 mmHg, and CL-hi by about 11 mmHg.

Evidently, "Sustained delivery of latanoprost by contact lenses is at least as effective as delivery with daily latanoprost ophthalmic solution. More research is needed to determine the optimal continuous-release dose that would be well tolerated and maximally effective. Contact lens drug delivery may become an option for the treatment of glaucoma and a platform for ocular drug delivery (Ciolino et al., 2016)"

Additional anti-glaucoma contact lenses that are currently being researched, synthesized, and patented are those that contain timolol maleate or brimonidine tartrate within a polymeric hydrogel. Timolol maleate belongs to a class of anti-glaucoma drugs known as beta-adrenergic blockers and brimonidine tartrate is an alpha agonist. Both are prescribed for cases of open-angle glaucoma. Specifically, in the case of beta blockers, systemic side effects can be pretty severe such as, cardiac arrhythmias, bronchospasm, and stroke and is therefore prescribed based on a patient's complete medical history. The aim of the study was to develop contact lenses that maintain normal hydration and comfort, and will dispense lower doses of drug for extended periods of time. This will lead to increased patient compliance, decreased negative side effects, and efficacious treatment (Schultz and Mint, 2002).

Etafilcon contact lenses (hydrogels) were washed in a saline solution and briefly dried. Then they were immersed in either a dilute solution of brimonidine tartrate (0.02%), or a dilute solution of timolol maleate (0.05%). (Topical ophthalmic solutions of the above drugs are commercially available as 0.2% solutions for brimonidine, 0.25% for timolol, and 0.5% for timolol ophthalmic gel forming solution). The lenses were subsequently tested on multiple patients as a replacement for their current regimens of eye drops. Instead of the patient administering their daily eye drop, he wore the contact lens for 30 minutes each day. In all cases, this method allowed for IOP to remain below the necessary value of 20 mmHg, with no evidence of ocular toxicity.

An additional study was conducted on glaucomatous beagle dogs. NIGHT & DAY™ silicone hydrogel contact lenses were immersed in timolol and phosphate buffered saline solution. Then, one lens was inserted into one of the dog's eyes, while the other eye served as the control, and no lens was inserted. The lenses with similar dosing to timolol eye drops led to an IOP reduction of about 5 mmHg (which is slightly greater than the IOP reduction resulting from timolol eye drops). However, lenses with a third drug loading as the eye drops led to a similar reduction in intraocular pressure, suggesting increased bioavailability. Finally, the eye without the contact lens remained unaffected by its proximal lens, which suggests reduction in systemic absorption of the drug released by the lens (Peng et al., 2012).

Fungal Keratitis

Fungal keratitis is an infection of the cornea (the clear, round dome covering the eye's iris and pupil) which causes pain, reduced vision, light sensitivity, and tearing or discharge from the eye. Resulting from infection from contact lens use, or from injury to the eye, fungal keratitis usually develops very quickly, and if left untreated, can cause blindness (Boyd, 2015). Fungal keratitis is also prevalent in tropical and subtropical climates (Ciolino et al., 2011).

Current treatment options for fungal keratitis vary depending on the severity of the condition. Topical eye drops are often the first line of treatment (Ciolino et al., 2011). Once again the above drawbacks to eye drops are present:

> "The failures of topical antimycotic treatments may be related to the limitations of eye drops as a form of drug delivery. Eye drops generate a transiently high concentration on application followed by a short period of effective therapeutic concentration and then a prolonged period of underdose. Furthermore, each drop is diluted

> and washed away by reflex tearing and dispersed by blinking. As a consequence, only 1% to 7% of drug in a drop is absorbed in the eye. The cornea absorbs only a fraction of this dose, in part due to the tissue's short contact time with the topical drops (Ciolino et al., 2011)."

Currently there is only one drug available on the market, natamycin, as a topical ophthalmic antifungal. However, this drug specifically is shown to have poor corneal penetration and is mainly effective with superficial corneal infections caused by Fusarium species (Singh, 2015). Depending on the severity and identity of the disease, often subconjunctival injections of an antifungal agent are prescribed and the dosage times are not infrequent, (twice every hour for the first 24 hours, then once every hour for the next 24 hours etc). "Successful antifungal therapy for fungal keratitis requires frequent drug administration for prolonged periods (ie, at least 12 weeks) (Singh, 2015)." Sometimes antifungals in an oral form are prescribed. However, 15 to 27 percent of patients with fungal keratitis require surgical intervention (Boyd, 2015). Even after surgery, a course of topical drops is often prescribed as well. Finally, surgery is not effective in all cases, and a patient may be rendered significantly visually impaired (Singh, 2015).

Research for an Anti-Fungal Contact Lens

The ineffectiveness of the topical regiment arises from low penetrance of the drug to the corneal epithelium as well as inadequate contact time between drug and tissue. Additionally, low patient compliance is common due to the frequency with which the drug needs to be administered. A contact lens that dispenses antifungal particles could resolve all these issues. A prototype antifungal contact lens (Ciolino et al., 2011) was synthesized using the following method:

Econazole, an antifungal drug, was added to a film of poly (lactic-co-glycolic) acid (PLGA). PLGA is desirable because of its biocompatibility and biodegradability, and its effectiveness at controlling drug release kinetics. Various film sizes were synthesized and all were encapsulated into polyhydroxymethacrylate (pHEMA), a common contact lens material. Contact lenses were synthesized with different concentrations of econazole. A control lens was created as well which contained the PLGA film inside the pHEMA hydrogel without the econazole. The contact lenses were tested against the fungus C. albicans, a common agent of fungal keratitis. First the lenses were placed directly onto a rich medium, a culture plate containing 1 mL of the Candida suspension. After a number of cycles of incubation and refreshing the medium, the culture was diluted, incubated, and counted for viable colonies. This was done to determine the effect of the contact lenses in direct contact with the fungi. The lenses were also tested for their drug-releasing capabilities. The testing was conducted by immersing the lenses in a yeast nitrogen base medium and incubated. Then they were immersed in fresh medium every 24 hours. The yeast nitrogen base drug release medium was collected at different intervals, and diluted with new medium containing C. albicans. Once again after a period of dilution and incubation, the suspension was plated and counted for viable colonies.

The results showed that both methods were capable of killing 100% of fungi for extended intervals. The release medium which contained contact lenses with 16 mg of econazole (PLGA-16) killed 100% of fungi for 21 days! The mediums from contact lenses, containing lower concentrations of econazole, killed fungus for shorter amounts of time. In the cases where the contact lenses came directly in contact with the fungal suspension, 100% of fungi were killed for 8 to 10 days (with PLGA-16). Studies show that C. albicans is more difficult to kill than Fusarium species. Therefore if Candida was killed by econazole, Fusarium should be as well (Ciolino et al., 2011). (Currently econazole is not FDA approved for ophthalmic use, although many ophthalmologists would prefer to treat fungal infections with something other than Natamycin, currently the only available drug).

Contact lenses that could elute the drug natamycin have also been synthesized (Phan et al., 2013). The study focused specifically on manipulating various contact lens materials. Hydrogels were composed of:

1. 100 % pHEMA,

2. 85% pHEMA and 15% [Tris(trimethylsiloxy)silyl]-propyl methacrylate (TRIS)

3. 75% pHEMA and 25% TRIS

4. N,N-dimethylacrylamide (DMAA),

5. 85% DMAA and 15% TRIS

6. 75% DMAA and 25% TRIS

The lenses were monitored by their uptake and release of two forms of natamycin. The first form was Natamycin dissolved in deionized water, and the second form was Natamycin encapsulated within poly(D,L-lactide)-dextran nanoparticles. Results indicated that the optimal materials to use were those containing DMAA. Furthermore, all gels had a greater uptake with the nanoparticles of natamycin versus natamycin alone. Finally, the release of natamycin within nanoparticles was greater than the natamycin alone. Also, the first hour of release was noteworthy.

The lenses with regular natamycin released 28-82% within the first hour. In the nanoparticle lenses this was reduced to 21-54% (Phan et al., 2013).

Hay Fever and Allergic Conjunctivitis

"Spring allergies are triggered as trees start blooming and billions of pollen grains are released into the air, causing susceptible individuals to develop allergic rhinitis… In these patients, the pollen causes degranulation of mast cells, which contain inflammatory mediators, ie, histamine and other allergy-causing chemicals. This process is clinically represented by sneezing; red, tearing eyes; postnasal drip; sinus headaches; feelings of sinus fullness; and itchy, scratchy throat (Medscape, 2016)."

As the conjunctiva of the eye is a mucosal membrane, it too is subjected to the inflammatory responses of allergic rhinitis. Per the CDC (Center for Disease Control and Prevention), approximately 19 million adults and another 6 million children suffer from hay fever in the U.S. alone. For the ocular symptoms of rhinitis, also known as allergic conjunctivitis, eye drops containing anti-histamines and/or mast cell stabilizers can be prescribed or recommended. Another class of treatments are corticosteroids and glucocorticoids, which also work to reduce the allergic reactions which affect the eye and relieve the negative symptoms. These can be prescribed as an ophthalmic suspension for topical use. Once again due to inhibitive pre-corneal factors, an alternate drug delivery mechanism is currently being researched in the form of a drug dispensing contact lens.

Research for DDCLs for Allergic Conjunctivitis

The Kingston University London conducted an in vitro experiment to synthesize a contact lens that could dispense nanoparticles of a synthetic glucocorticoid, prednisolone, into the eyes of patients with allergic conjunctivitis. (Currently prednisolone is on the market as prednisolone acetate ophthalmic suspension for topical use.) The main purpose of the experiment was to consider the effects of the encapsulated drug on the contact lenses' functionality and safety as well as the drug's bioavailability (ElShaer et al., 2016).

Prednisolone nanoparticles (PNP) were synthesized using an emulsion-solvent evaporation method. The experiment was designed to maximize three key nanoparticle features: small particle size (increased surface area/bioavailability), highest encapsulation efficiency, and maximum surface charge (no coagulation of particles; increases stability). To obtain the smallest particle size, four variables were manipulated: PLGA (poly-lactic-co-glycolic acid), PVA (polyvinyl alcohol), API (amount of prednisolone used), and homogenization time. Through optimization of these components a particle size of about 295 nm was obtained. To form the contact lens molds, HEMA (2-hydroxymethacrylate), MAA (methacrylic acid) and a small amount of EGDMA (ethylene glycol dimethacrylate) were mixed together along with the PNPs. These four hydrogel materials were allowed to polymerize thermally for 4 hours at 80°C in molds of polypropylene.

The in vitro drug release pattern of the contact lens with 0.4 grams of PNP was observed to be a two-phase process: an initial burst, followed by a period of slower release. The lens was placed in a release medium of phosphate buffered saline for 24 hours. 10.8% of drug was released in that time. The slow release of the drug can be due to the need for the drug to get past its nanoparticle barrier and through the contact lens as well. One of the issues with eye drops is that all of the drugs are released within a few hours. Nanoparticles of medication embedded in contact lenses can provide a longer lasting therapeutic regimen (ElShaer et al., 2016).

Method of Drug Release from Contact Lens

Although some studies for drug-eluting contact lenses pre-soak the contact lenses in drug, to allow for eventual diffusion into the eye, to achieve a more controlled method of release, other methods are being researched. The human tear film contains an enzyme called lysozyme. In a study on anti-glaucoma contact lenses, timolol maleate was encapsulated in nanodiamond (ND) particles. The NDs were coated in both polyethyleneimine (PEI) and chitosan. Chitosan is an enzyme-cleavable polysaccharide and PEI enables a more effective cleavage. The drug release of these impregnated lenses was monitored in vitro. In the absence of lysozyme, no release of timolol maleate was detected. In the presence of lysozyme the lens released 9.41 micrograms in 24 hours (Kim et al., 2014).

Addition of Drug to Contact Lens Material and Subsequent Hydration and Oxygen Permeability

This feature was monitored in the prednisolone study cited above. Contact lenses lacking the PNPs had an average hydration of about 36%. Lenses containing a smaller volume of drug nanoparticles (0.2 g) had a decreased hydration by about 31%, whereas the lenses with a higher volume of PNPs (0.4 g) had a further reduction in hydration to about 30.5%.

Surface wettability determines comfort of the lens, and was measured as well. A good surface wettability is identified by a contact angle less than 90 °C. Unmodified lenses have a contact angle of 85 °C. The prednisolone encapsulated lenses had further reduced angles which should increase ocular comfort (ElShaer et al., 2016). Similarly, contact lenses containing nanodiamond particles of timolol maleate demonstrated acceptable hydration values (Kim et al., 2014).

Transparency/light Transmission Capability of Drug-Impregnated Lens

Ideally a contact lens should have a light transmittance of above 90%, so vision is unobstructed. The control contact lenses (lacking PNP) in the prednisolone study had a high transparency of 94.5%. The lenses containing 0.2 grams and 0.4 grams of PNP had a reduced transparency of 86.23% and 83.1% respectively. However, this amount contributes to low or no opacity, and as long as the correct amount of nanoparticle is added to the lens, vision should not be compromised (ElShaer et al., 2016). Similarly, addition of nanodiamond particles of timolol maleate to a pHEMA lens did not cause any discernable changes to the lens' optical clarity. The lens with a higher concentration of NDs maintained a transmittance of 84.5% (Kim et al., 2014).

Dimensions/measurements of Drug-Eluting Contact Lenses Compared to Commercially Available Lenses

In the study done on antifungal contact lenses, when synthesizing the econazole-laden lenses, parameters of an 8.05 base curve and a 15.5 mm diameter were measured, which are consistent with commercially available lenses (Ciolino et al., 2011).

Preservation of Contact Lens through Lyophilization (to prevent drug elution/ degradation) Effect on Lens Capability

Depending on the method used to impregnate the lenses with drug, there exists a risk of the drug eluting out of the lens during storage. In order to combat this, anti-fungal contact lenses were lyophilized, a preservation process involving the freeze drying of a substance and subsequent removal of water by a vacuum causing the water to go from an ice state directly to a gaseous one. The fungicidal activity of the lyophilized lens was then assessed and found to be intact, although the duration of its effectiveness was reduced by 1 to 2 days (Ciolino et al., 2011).

Risk Factors and Drawbacks Associated with a DDCL

Although there is a lot of potential is this innovative drug delivery system, several potential downsides should be noted. There are many consumers who do not wear contact lenses because they find them uncomfortable or haven't found the proper fit. Others do not wear contact lenses because they have no refractive error and would thus need a special fitting session just to wear a short-term lens. Additionally, glaucoma often affects the geriatric population. Individuals of this population could also have difficulty inserting and removing the lenses, however this issue could be aided by an eye-care professional. These factors could potentially minimize the market for such lenses. Another problematic feature involves the drug-eluting property of the lenses. Once removed by the patient any remaining drug may continue to diffuse out. In the case of anti-fungal drugs, this could have an effect on the development of resistant strains while in the case of other drugs this may simply pose as a hazard for children. (It should be noted though that with lenses controlled by lysozyme presence this undesired drug-elution may be minimized).

Conclusion and Further Applications

Contact lenses for the treatment of glaucoma, fungal keratitis, and allergic conjunctivitis have been synthesized and demonstrate much potential in effective treatment. However, the lenses are far from having a clinical relevance. Much more animal and human testing is required prior to the necessary FDA-type approvals. Although in this paper glaucoma, fungal keratitis, and allergic conjunctivitis were discussed, research is also underway for additional ocular conditions such as, chronic dry eye and bacterial infections (Legett, 2009), (ElShaer et al., 2016). Additionally, the studies are working on embedding various drug nanoparticles into lenses without obstructing optical transparency. Potentially, instead of drugs, various ocular-necessary vitamins and supplements can serve as the embedded nanoparticle. As salt is iodized to promote proper thyroid function, perhaps macular degeneration could be prevented by infusing contact lenses with nanoparticles of lutein and zeaxanthin, two nutrients vital to a healthy macula.

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Mechanical Factors Affecting Heart Morphogenesis

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Abstract

For years, the genetic element of heart morphogenesis has been studied. This review focuses on a relatively new area of study, namely, mechanical factors influencing heart morphogenesis. To understand the context of the role of mechanical factors in heart development, an extensive review of the stages of heart morphogenesis is provided.

It is found that shear stress, surface tension, fluid forces, and contractions of certain cells play a role in various stages of heart development. Numerous studies have shown that in cases where these mechanical forces were modified, abnormal heart defects were produced. These studies prove that the mechanical forces are essential for normal heart morphogenesis.

Although attempts have been made to define the mechanisms involved in these pathways, most of the research done so far has been inconclusive. While it has been proven that the mechanical forces play a role in heart development, it is still unclear exactly how the forces are involved in the developmental pathways, and what initiates them to proceed. As of current studies, it is also still unclear if there is any correlation between the genes and the mechanical factors involved in heart morphogenesis.

Introduction

One of the most widely studied biological topics is the field of morphology. Specifically focusing on embryonic morphology, much research has been done to link genetics with the various formations and stages of embryonic development. Throughout the years, genetics has been used to explain and account for countless aspects of development patterns and pathways found in embryonic development. Many genes and signaling molecules have been identified and used to formulate blueprints of morphogenic pathways in a developing embryo.

In recent years, there has been a shift in the study of embryonic development. New studies are beginning to focus on the possible role of mechanical and physical forces in morphogenesis. There are numerous physical forces found within the embryo during the stages of development. This review focuses on contractions of cells, fluid forces, shear stress, and surface tension, and their impact on cardiac morphogenesis. Breakthroughs and new technology in mechanical biology allowed for advanced studies of these forces.

Finally, the data will be analyzed to determine if these physical forces that are involved in cardiac development can potentially be linked to genetic factors. Recent studies have begun to attempt to relate the mechanical factors affecting heart morphology with the genetic factors which have been studied for many years. By bridging the gap between genetic and mechanical forces, we will have a better understanding of the control of the development of cardiac tissue and the heart within an embryo.

Methods

Critical analysis of peer reviewed journal articles and original clinical research papers was used to write this review. The articles and papers from which the research was gathered were obtained by using the PubMed search engine found on the government's National Center for Biotechnology Information website. Additional references were obtained from those sources. Keywords used were heart morphology, heart development, mechanical forces, and hemodynamics.

Heart Fields and the Heart Tube

Before analyzing mechanical factors involved in embryonic cardiac morphology, it is essential to have a solid understanding of the stages of heart development in an embryo. The current accepted model of cardiogenesis was first discovered during the last decade. Heart morphogenesis begins with cells with myocardial potential that are located in a specific region, known as a heart field (Buckingham et al., 2005). This was the first research done which classified two individual heart fields, both of which participate in cardiac development. Before this, there were others who identified a second heart field (Kelly et al., 2001). However, Buckingham was the first to explain the process by which the two different fields give rise to distinct portions of the human heart.

Much of this research was done with chick embryos and mouse embryos. Comparisons of gestation timelines of chicks or mice to humans can be made using the formulas outlined by Srivastava (Srivastava, 2006). The anterior lateral plate mesoderm gives rise to cells in the first heart field. A crescent shape is formed by the first heart field. This occurs at approximately the second week during human gestation. By the third week the ends of the crescent merge with each other, forming a basic heart tube. This heart tube is considered basic because it is simply made up of two layers: an interior endothelial cell layer, and an exterior myocardial cell layer (Srivastava, 2006).

Simultaneously, the second heart field is expanding in a formation which appears to encircle the first heart field. The first heart field in the basic heart tube acts as a platform upon which the second heart field is able to settle and begin the process of becoming the various chambers found in the heart (Buckingham et al., 2005). Figure 1 labels the heart fields and shows how each field ultimately gives rise to a different portion of the heart.

It also gives a clearer understanding to the anterior/posterior positioning of the first heart field (labeled "FHF") and the second heart field (labeled "SHF") at two and three weeks of human gestation. It depicts how the second heart field (shown in yellow) uses the first heart field (shown in red/pink) as a support in the initial stages of morphogenesis. Figure 1 also clearly illustrates how the second heart field completely surrounds the first heart field by three weeks of human gestation.

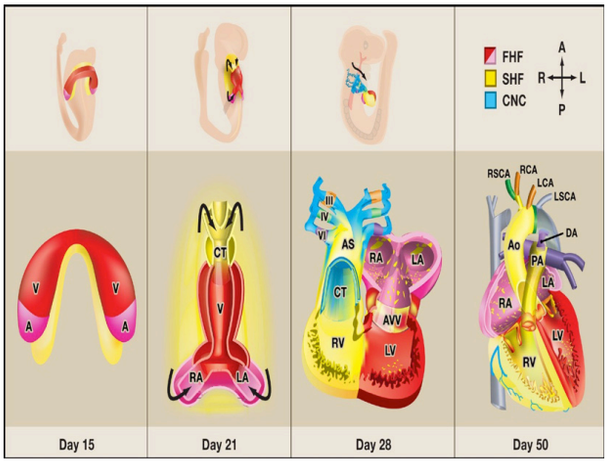

Figure 1. Mammalian Heart Development (Srivastava, 2006).

It is unclear what exactly causes the crescent shaped first heart field to bend into a heart tube. It is theorized that contraction of the crescent, caused by microfilaments in the apical regions of the epithelial cells results in the production of the tube. The contractions force the cells to assume a wedge-shape, causing the plane of the cell sheet to begin to bend into a tube-like formation (Taber, 1998). Unrelated research supported this theory by reporting that administering pharmacological agents that inhibited actin filament function prevented the heart tube formation and resulted in the persistence of the crescent shape (Ettensohn, 1985). Because no experimental work has definitively linked contractions of the actin microfilament with the crescent bending and heart tube formation, it would be inappropriate to say that this is definitely the mechanism which forms the heart tube. Nonetheless, the research provided is enough to strongly suggest that this is the correct method of heart tube formation.

Dextral Looping

Before the heart can fully develop into its various components and chambers, it must go through an event known as looping. Looping produces the c-shaped structure present in the third segment of Figure 1. There are various stages of this looping process, also known as "dextral looping." To analyze this process research was performed using chick embryos. Before looping begins, in a stage referred to as the prelooping stage, the heart tube is essentially bilaterally symmetrical. As the heart tube undergoes rapid elongation, interventricular grooves are formed. These grooves ultimately become the bending points upon which the bottom curve of the "c" looping is established. Upon the completion of c-looping, three bended bands are easily noticeable: the truncus arteriosus (which will later develop into the aorta, its branching arteries, and the pulmonary trunk), the primitive atria region, and the primitive ventricular region (Manner, 2000). Figure 2 gives detailed pictures of the process of dextral looping.

It can be seen clearly in Figure 2 that throughout the process of dextral looping, the heart loses the symmetry and linearity it possessed during the previous stages of the heart tube (Manner,

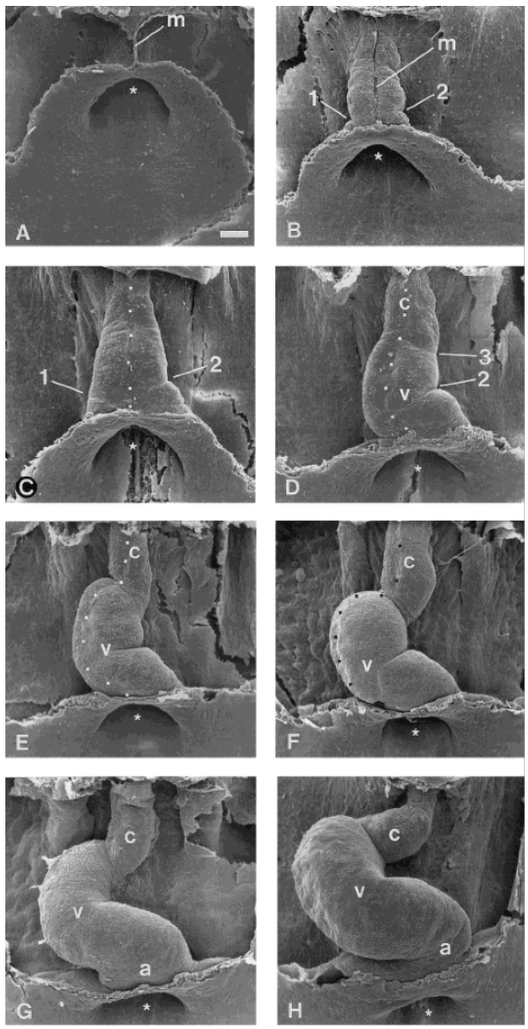

Figure 2. Stages of Dextral-Looping. Slides A and B represent the prelooping stage. In Slide B, "1" and "2" represent the interventricular grooves. Slides C – G show the incremental changes in the heart tube as it proceeds through the process of dextral looping. In Slide H, upon completion of dextral-looping, "c" represents the conus, "v" represents the primitive ventricular region, and "a" represents the primitive atria region (Manner, 2000).

2000). The conus will ultimately give rise to the aorta, the left and right subclavian arteries, the left and right carotid arteries, and the pulmonary trunk. The primitive ventricular region will ultimately give rise to the left and right ventricles. The primitive atria region will ultimately give rise to the left and right atria (Srivastava, 2006).

While the slides in Figure 2 show actual images of the stages of dextral looping, the third segment in Figure 1 diagrams how the developing heart appears after dextral looping of the heart tube is completed. Upon completion of looping, the aorta and its various branching arteries are not fully developed. The aorta appears as a simple structure known as the aortic sac (labeled AS in Figure 1). At this stage of development, the branching arteries of the aorta (left and right subclavian arteries and left and right carotid arteries) are bilaterally symmetric branches (labeled "III" and "IV" in Figure 1) stemming off of the aortic sac. As Figure 1 illustrates, following dextral looping, the aortic sac and its branches are not yet positioned between the atria (Srivastava, 2006).

Transformation of C-Shape to S-Shape

Following dextral looping, the heart goes through another morphogenic process, converting the c-shaped heart loop (created by dextral looping) into an s-shaped heart loop, resulting in the placement of the ultimate locations of the chambers and modifying the position of the craniocaudal axis. This process pushes the right atrium to be positioned superior to the right ventricle, and drags the left atrium towards the right atrium, aligning the left atrium almost directly above the left ventricle. This process causes the aortic sac and its branches to become positioned between the atria. Because the ultimate location of the aorta will be parallel to what will eventually be the interatrial septum,

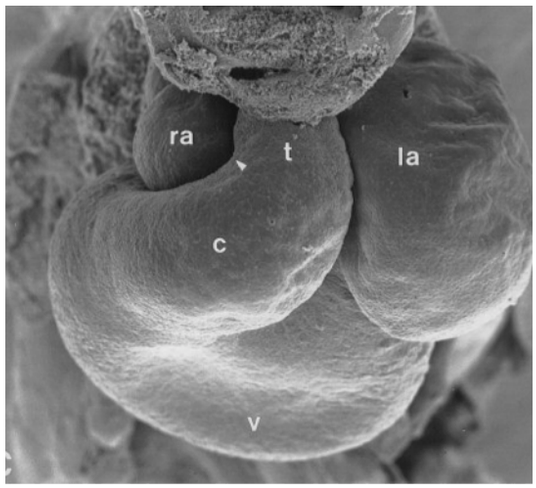

Figure 3. S-Shaped Heart Loop. The left and right atria are represented by "la" and "ra", respectively, the ventricles are represented by "v", and the aortic sac is represented by "c" and "t" (Manner, 2000).

this positioning of the aortic sac and its branches between the atria is essential for the process of heart morphogenesis to proceed (Manner, 2000). Figure 3 shows the resulting s-shaped heart loop with the aortic sac positioned between the atria, which have become aligned superiorly to the segment destined to be split into the left and right ventricles.

Blood Flow during Heart Formation

By the time that looping is completed, there is already blood flow present in the developing heart. At this point, septation of the atria into right and left components has not yet occurred. The blood flows from the portion of the primitive atria region destined to be the left atrium down into the primitive ventricular region. It flows through an area known as the AV canal, the walls of which will ultimately give rise to the region of the atrioventricular valve. Because septation of the ventricles has also not yet occurred, the blood must flow through the primitive ventricular region with enough force to navigate the entire ventricular loop. The blood enters the primitive ventricular region via the inlet tract, ultimately destined to become the left ventricle, and exits the ventricular loop via the outlet tract, ultimately destined to become the right ventricle. The inlet and outlet tracts are delineated by the interventricular foramen, which is an opening separating what will ultimately be the left and right ventricles. As will be described later, septation of the ventricles occurs by the closing of the interventricular foramen (Moorman et al., 2003).

Development of the Cardiac Chambers (Septation)

Changes in the atria and ventricles occur over the same period of time. As the developing lung forms, a network of vessels surrounds the lung buds. This network of vessels connects with the primary atrium (the unseptated atria) and attaches at a point on the left portion, in its inferior region. (This network of vessels, ultimately destined to become the pulmonary veins, does not expand and become fixated on the roof of the left atrium until after septation is completed.) In the right portion of the primary atrium, the sinus venous, the small cavity where the superior and inferior vena cava drain, attaches to the right atrium.

As this group of vessels attach to the primary atrium, the left and right atrial appendages begin to grow out of the walls of the primary atrium. The appendages are sacs which form in the muscular walls of the left and right atria. While the right atrial appendage is quite large and expands distally in a continuous path parallel with the right atrium, the left atrial appendage is narrower, and positioned in the superior portion of the left atrial wall. This is because the formation of the left atrium consists of a much larger portion of the primary atrium. The appendages are notably the first appearance of morphological differentiation

between the left and right atria. Known as morphological sidedness, this development is controlled by a pathway governed by the Pitx2 gene (Moorman et al., 2003).

As all of this is occurring, septation of the atria is also progressing. Cardiac jelly found in the edges of the region of the septum begin to fuse into ridges, eventually giving rise to an almost complete atrial septum dividing the primary atrium into the left and right atria. Blood resists entering into pulmonary circulation because the lungs do not become inflated until the baby is born. The foramen ovale is an opening found in the atrial septum. The foramen ovale allows for the blood in the right atrium to shunt into the left atrium, avoiding pulmonary circulation. It is formed by the overlapping of the two bands of the septa, resulting in a unidirectional valve (Bressler, 1990). At birth, the foramen ovale closes, and is represented in the adult heart as the fossa ovalis. Figure 4 shows a drawing of the embryonic heart with the foramen ovale. It is clear from the diagram how the foramen ovale is formed by the overlapping of the two bands of the septa.

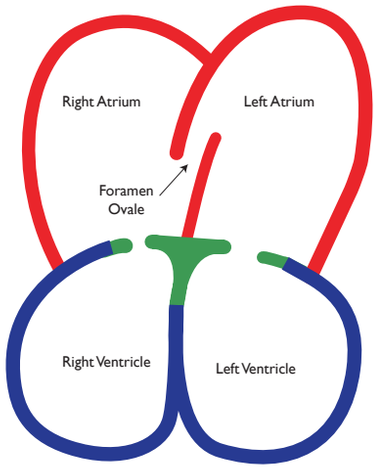

Figure 4. Diagram of Foramen Ovale Shunting Blood from the Right Atrium to the Left Atrium

Simultaneously, development of the interventricular septum is occurring. By the time that heart tube looping finished, the primary interventricular foramen (which connects the left and right ventricles before the interventricular septum develops) becomes noticeable (Moorman et al., 2003). The dextral loop is made up of an inner curve and an outer curve. The outer curve balloons out, extending the ventricular wall and increasing the size of the lumen (Davis, 1927).

Numerous studies have attributed ventricular development to consolidation of myocardial trabeculae found in the primitive ventricular region. Myocardial trabeculae are spongy jagged folds of endocardium running along the circumference of the primary heart tube (Icardo, Fernandez-Teran, 1987). As these jagged edges become more compact and consolidated, the ventricular walls are formed (Moorman et al., 2003). Unfortunately, due to the geometric randomness and inconsistencies of the folding and compacting of ventricular trabeculae, so far no one has been able to successfully produce an accurate and definitive model of the methods involved in ventricular trabeculation (Taber, Perucchio, 2000).

Valvulogenesis

As the heart chambers undergo morphological pathways to attain their final shape, sizes, and orientations, the atrioventricular valves are forming. In the right side of the heart, the atrioventricular canal expands in order for the right atrium to become continuous with the right ventricle. During this process, the muscle tissue found in the right half of the atrioventricular canal becomes integrated with the right atrium, forming the region where the tricuspid valve will develop. In the left side of the heart, the left atrium is already continuous with the left ventricle due to a mechanism which occurs during looping. Similar to the right side, the muscle tissue found in a portion of the atrioventricular canal becomes integrated with the left atrium, forming the region where the mitral valve will develop (Moorman et al., 2003).

The function of the heart valves is to prevent backflow of blood from the ventricles into the atria. The valves are often described as multileaflet structures (Bartman, Hove, 2005). There is an abundance of a gelatinous substance known as cardiac jelly found in between the endocardium and the myocardium. It is believed that as the cardiac jelly in specific regions of the heart begins to swell, endocardial bulges are formed. These cushions are further enhanced by migrating cardiac endothelial cells, changing the cardiac jelly into cardiac mesenchymal cells, causing the endocardial bulges to begin to grow. Once their growth is completed, they are referred to as endocardial cushions (Markwald et al. 1977). These endocardial cushions then proceed through another stage of growth, ultimately forming heart valves made of fibrous tissue. The fibrous tissue found in the heart valves allows for them to open and close as the heart pumps blood throughout its chambers (Bartman, Hove, 2005).

The pulmonary valve and aortic valve have similar functions to the atrioventricular valves. They prevent backflow into the right ventricle and left ventricle, respectively. The mechanism for their morphogenesis is very similar to the growths of endocardial cushions described above in the formation of the atrioventricular valves (Bartman, Hove, 2005).

Development of the Arterial Trunks

Throughout heart development, the aortic sac undergoes changes to become the outflow tracts of the heart. As the distal outflow portion of the original ventricular loop begins to

extend out of the pericardial cavity, it is divided into two, forming the ascending aorta and the pulmonary trunk. The ascending aorta and the pulmonary trunk are two separate tubes, divided by the aorticopulmonary septum. Figure 5 shows a scanning electron micrograph image of the bend formed in the tube. The edge of the distal outflow portion of the original ventricular loop completely extends to the edge of the pericardial cavity. The distal outlet portion of the aortic sac develops into the distal portions of the aorta and its branching arteries, completing the systemic circulation path. The pulmonary trunk extends and attaches to the lungs to complete the pulmonary circulation path (Moorman et al., 2003).

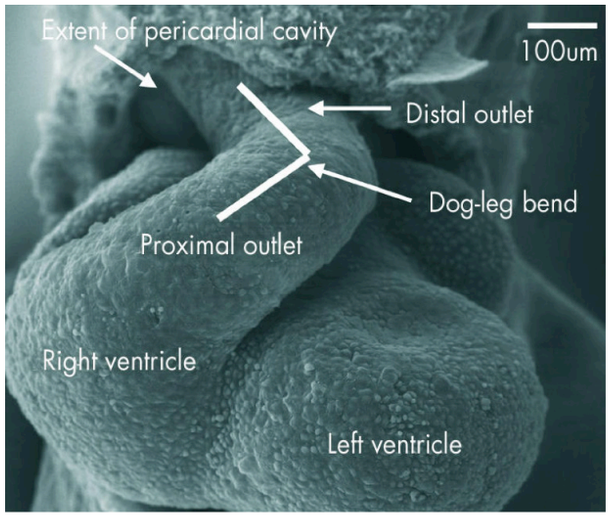

Figure 5. Bending of the distal outflow portion of the ventricular loop (Moorman et al., 2003).

As can be seen in this image, the dog-leg bend produced by the outflow tract helps align the aorta with aortic arches III and IV, and the pulmonary trunk with arch VI (Moorman et al., 2003).

Mechanical Forces Involved in Heart Morphogenesis

When analyzing mechanical factors affecting heart morphogenesis, the initial, and probably most striking piece of data, is that the heart begins to function before it is completely developed. Blood begins to flow through the primitive heart chambers by the time that initial stages of heart looping are completed. This suggests that the actual pumping of the heart, along with other forces produced by blood flowing through the heart chambers, might actually be the cause of later steps of heart morphogenesis (Bartman, Hove, 2005).

One of the accepted mechanisms of heart tube formation involves forces caused by contracting actin microfilaments. The forces caused by the contractions mold and bend the crescent into a tube shape, forming the heart tube (Taber, 1998).

Many theories have been produced to try to link biomechanics with heart looping. As early as 1970, a theory was suggested that looping is caused by differential growth of portions of the heart tube. Differential growth is when cells of the same structure grow at different rates, causing the structure to undergo a physical change of shape and other physical properties (Stalsberg, 1970). Others suggested that heart looping is caused by pressures placed on the heart tube due to the expanding cardiac jelly (Manasek et al., 1984). Later scientists proposed that looping is caused by residual stress which causes the dorsal mesocardium to shorten and bend (Taber, 1995). Although these theories give possible mechanisms for the cause of heart looping, they do not provide a clear explanation for what causes the heart tube to bend in a specific orientation.

Additionally, all of these theories are based on the assumption that the forces controlling heart looping are forces from within the heart tube (Bartman, Hove, 2005). In 2002, an experiment was run to attempt to prove that heart looping is caused by forces placed on the heart tube from external adjacent tissues pressing on the embryo. The splanchnopleure is an extraembryonic membrane which presses against the heart tube on its ventral side. Because heart looping involves ventral bending and rightward rotation, the forces applied by the splanchnopleure assist the heart in rotating in the rightward direction. In an experiment, the splanchnopleure was removed from 24 chick embryos. In all 24 cases, abnormal heart looping was reported, proving that forces applied by the splanchnopleure have a direct effect on heart looping. In most of the cases, slightly skewed ventral bending was reported, along with reports of minimal rightward rotation of the heart tube.

In most ex vivo experiments that analyze heart development, such as this one, conditions required to analyze the growth are dependent on factors which cause surface tension. Under normal in vivo conditions, surface tension forces have not been found to affect heart looping. It is interesting to note that in a parallel experiment, it was found that by placing the chick embryos in a liquid medium and thereby eliminating standard ex vivo surface tension forces, some abnormalities were found with respect to heart looping. This suggests that there are in fact surface tension forces present in vivo which play a role in heart looping (Voronov, Taber, 2002).

Another pathway regulating heart looping begins with secreted proteins flowing through the heart tube. Although very little is known about this pathway and the forces involved, it is known that it in some manner provides the forces required to initiate rightward rotation of the heart tube. This pathway is controlled by the Pitx2 gene, the nodal gene, and the lefty gene (Moorman et al., 2003).

Myocardial trabeculae are known to have many effects on the developing heart. While they are mainly known for their assistance in helping blood flow in the appropriate direction prior to formation of the atrial and ventricular septa, myocardial trabeculae also produce peristaltic contractions within the myocardium. These contractions help the trabeculae to become more compact and to fold along each other, thereby producing the ventricular walls (Thompson et al., 2000). A result of producing and solidifying the ventricular walls is that the intramyocardial blood flow increases. This increase in blood flow will cause an increase in the fluid forces affecting heart morphogenesis, and will also cause these stresses to be more evenly distributed within the portions of the heart upon which they are working (Taber, 1998).

The role of hemodynamics in septation has been debated for many years. The "flow molding" hypothesis suggests that cardiac septa form due to opposite pressure gradients formed by two antiparallel streams of blood flowing through the heart simultaneously (Jaffee, 1963). Scientists who support the flow molding hypothesis believe that the cells lining the heart tube are able to detect changes in pressure caused by different flow velocities and directions. When such changes are detected, morphological changes are induced, causing the septa to form. Using this biomechanical mechanism as a springboard, they also suggested that these fluid forces assist in shaping and molding the cardiac jelly into firm structures in order to form the walls of the heart chambers (Dewey et al., 1981). Furthermore, experiments proved that modifying the fluid velocities in developing chick hearts can result in many heart malformations, including deformed ventricular septum formation (Clark et al., 1989). Challengers of the flow molding theory argue that the whole foundation of the theory is incorrect. They have used advancements in imaging technologies to show that the initial blood flow through the heart chambers does not appear as two antiparallel streams. This data suggests that perhaps septa formation is not at all affected by fluid forces. Possibly, it is the formation of the septa which may actually create and direct the streams of fluid; the heart septa might be the initial cause of formation of certain fluid forces found in the heart (Yoshida et al., 1983).

The frictional force caused by fluid flowing along the surface of a cell is known as shear stress. In the developing heart, shear stress is caused by the blood which is being pumped throughout the developing chambers. Shear stress is known to play a very major role in valve formation. In a study done with zebrafish, reduced fluid forces resulted in complete prevention of valve formation (Hove et. al., 2003).

In a recent study, a positive displacement pump was used to simulate the fluid forces and shear stress produced in the developing heart. Because the experiment focused on valvulogenesis, embryonic chick hearts with endocardial cushions were isolated. They were placed inside a tubular collagen scaffold, and remained there untouched for 72 hours. The purpose of this 72 hour period was to guarantee that the cells adhered to the inside of the tube (Tan et al., 2012). It is known that hemodynamic forces increase as heart development progresses (Biechler et al., 2010). Therefore, the forces applied by the pump were divided into two periods, one of a low force, followed by one of a higher force. Obviously, the actual level of fluid forces and shear stress found in vivo could not be completely replicated, for had these forces been applied, the cushions would have been pushed out of the tubular scaffold. The forces used in the experiment were based on formulas used to determine the highest magnitude of force that the cushions would be able to withstand without being dislodged from the tube. Initially, through the use of a positive displacement pump, a force with a frequency of 5.38 x 10-2 Hz with an average velocity of 0.07mm/s was applied in the tube for a period of three days. The shear stress calculated based on these numbers is 7.6 x 10-4 Pa and a positive pressure of 4.3 x 10-3 Pa. After this three day period, the flow rate was raised to a force with a frequency of 0.29 Hz with an average velocity of 0.86mm/s, and was applied for an additional four days. The shear stress calculated based on these numbers is 9.4 x 10-3 Pa and a positive pressure of 0.053 Pa. The shear stress in the second flow phase is equivalent to a 92% increase. Control groups were set up in tubes where no pump was attached. The control tubes were attached to bioreactors to ensure a closed loop would exist, thereby maintaining comparable oxygen levels and other conditions with the flow tubes.

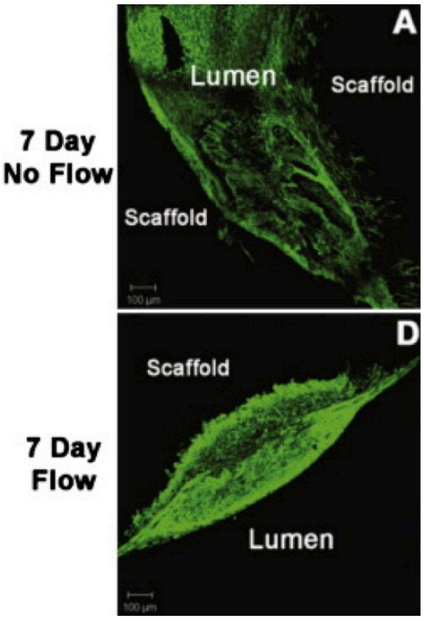

Figure 6. Comparison of flow and no-flow cushions (Tan et al., 2012).

Following the first (days 1-3) and second (days 4-7) phases of pumping forces, the "flow" cushions and the "no-flow" cushions were analyzed. After the first phase, no significant differences were found between the flow and no-flow cushions. However, analysis after the second phase revealed amazing results. The no-flow cushions formed a scattered network of cells in the lumen of the tube. This network was very loose, and did not exhibit signs of the rigidness or compactness normally found in heart valves. In contrast, the flow cushions formed a very compact mass of cells. The mass was wedge-shaped, and had leaflet-like structures extending off of it (Tan et al., 2012). Figure 6 shows confocal laser scanning microscope images comparing the cushions.

A signaling molecule, rhoA was also studied in this experiment. rhoA regulates the stiffening of the AV cushions, a prominent characteristic. The flow cushions were found to be much stiffer than the no-flow cushions. It was found that rhoA message levels were increased by flow forces. In addition to that, it was also reported that inhibiting a downstream effector of rhoA, rhoA coiled-coil containing kinase (ROCK), caused a decrease in stiffness of the fibers in the AV cushions. Figure 7 shows a graph comparing the expression of rhoA in flow and no-flow cushions (Tan et al., 2012).

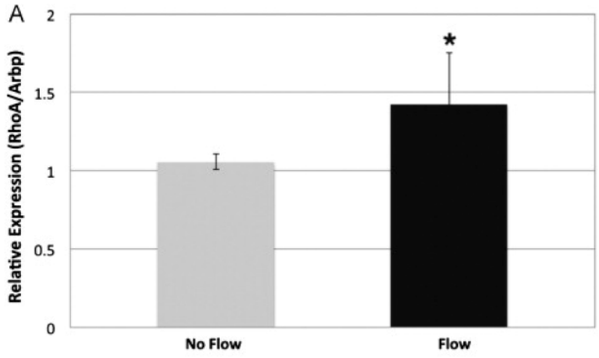

Figure 7. rhoA expression in flow and no-flow cushions (Tan et al., 2012).

This study was a major breakthrough in relating biomechanical forces to heart development. Even though the shear stress and fluid forces applied in this study were significantly below values found in vivo, the cushions and proteins still responded appropriately. The flow cushions still developed into wedge-shaped stiff masses, and rhoA was clearly upregulated.

Discussions

Formation and development of the human heart involve many precise and intricate steps. New studies have analyzed how many of these steps are initiated by mechanical factors. A major element of this hypothesis is that the heart begins to pump blood before it is completely developed. The fluid rushing through the developing heart causes shear stress, flow pressure, and surface tension. With experiments, these forces have been proven to play a role in propagating various stages of heart development, with specific focuses on heart looping and valvulogenesis.

Other mechanical factors, such as contractions and expansion of cardiac jelly, have also been linked to heart development. With regard to all of these forces, studies have been done to show abnormal heart development in experiments where the forces have been eliminated. This provides a strong basis for theories suggesting that these mechanical forces play a major role in heart development.

Because the study of mechanical factors affecting heart development is relatively new, much of the research is still inconclusive. While in many cases it has been proven that mechanical factors play a role in specific stages of heart development, much of the research has not been able to specify exactly which mechanical factors affect which steps of heart development. Additionally, the research done to date has not been able to prove, in many cases, if the mechanical factors involved are causes or effects of certain stages. Specifically with regard to septation, it has been debated for years whether the blood flow paths cause the septation to occur, or is a result of septation. Nonetheless, it has been documented that mechanical factors are definitely present in heart development, and regardless of whether they are the cause or effect of one step, they definitely affect propagation of further steps of heart development.

An interesting facet of studying the role mechanical forces play in heart development is to attempt to link these forces to the genes and genetic pathways which have already been discovered. Due to the relative novelty of studying mechanical forces in heart development, minimal breakthroughs have been made with respect to linking these forces to genetics. Although a few genes have been recognized as working simultaneously with mechanical factors, it is unclear whether these genes are influencing the mechanical factors, or are merely present at the same time and only affecting other, non-mechanical, elements of heart morphogenesis. Future studies need to be performed in order to determine whether or not these mechanical force are linked to genetic factors. If they are proven to be linked, future studies will be necessary to determine to what extent they are linked and to determine the exact mechanisms relating the genes to the mechanical forces. Relating mechanical factors to genetics could lead to major breakthroughs in diagnosing and preventing heart developmental abnormalities.

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The Effects of Sports Drinks on Teeth

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Abstract

With a push towards a more active lifestyle, the sports drink industry has grown substantially in recent years. However, despite their popularity, sports drinks contain acid, giving them extremely low pHs, which can cause erosion. There have been many studies, including self-administered surveys, studies in vitro, and studies in situ that have shown sports energy drinks cause dental erosion, leading to permanent loss of tooth volume and a softening of the outer layer of the tooth.

Introduction

Sports drinks were initially created for the purpose of rehydration and electrolyte replacement for athletes during intense physical activity. (Mathew et. al. 2002) The drinks have proven to increase concentration, stimulate metabolism, and eliminate harmful substances from the body (Pinto et. al. 2013). However, sports drinks have become popular today among amateur athletes and ordinary exercisers looking for a drink after a workout. With the push for a healthier lifestyle, more people have begun to exercise and many drink sports drinks on a regular basis. In 2000, the sports drink market was estimated at 1.2 billion dollars and has been growing since (Hooper et. al. 2005). Current research has demonstrated that these drinks have negative effects on teeth and can eventually lead to teeth rotting. With the growth in the sports drinks industry, it is critical to understand the effects sports drinks have on the body. This paper attempts to examine sports drinks from the perspective of oral health and review the current knowledge on the effects of sports drinks on teeth.

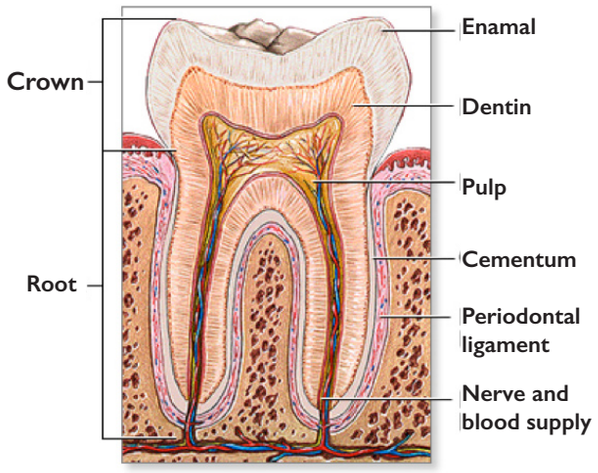

Figure 1. The anatomy of a tooth. The enamel being most superficial and moving deep, the cementum, dentine, and pulp. (https://medlineplus.gov/ency/images/ency/fullsize/11121.jpg)

Tooth structure

Teeth are composed of three different types of tissues: enamel, cementum, and dentin. The enamel is the most superficial of all three and is the hardest substance in the entire body. The enamel is composed of 96% minerals with the rest being water and organic content. The hardness and density of enamel both decrease further from the surface (Lussi et. al. 2011). Because the enamel comes into contact with the outside, it is the most affected by acid producing bacteria, which can cause dental caries. The cementum, which is avascular, is the part of the tooth that covers the root of the tooth. Collagen fibers project out of the cementum, forming most of the periodontal ligament, which keeps the tooth attached to in its socket. Deep to both the enamel and cementum is the dentin (Ross et. al. 2003). The dentin makes up most of the tooth and is in contact with the dental pulp. The dentine is made up of 47% minerals and 33% organic content (Lussi et al. 2002). This dental pulp is both highly vascularized and very innervated (figure 1).

Effects of Acid on Teeth

Dental erosion is the dissolution of tooth mineral caused by external sources and not from bacteria, like plaque (Arnauteanu et. al. 2015). It is characterized by initial softening of the enamel surface. This leads to further dissolution of the enamel crystals, which will cause permanent loss of tooth volume and a softened surface layer (Lussi et. al. 2002). Erosion, as opposed to tooth decay, leads to a widespread thinning of the tooth surface without causing dental caries (Milosevic 2004.) Erosion occurs when the pH of the solution around the enamel is lower than 5.5. At this low pH, the hydrogen ions dissolve the minerals which allows the calcium and phosphate ions to diffuse out of the teeth (Adhani et. al. 2015). In dentine only the mineral portion dissolves in acid while the organic component remains (Lussi et. al. 2011)(figure 2).

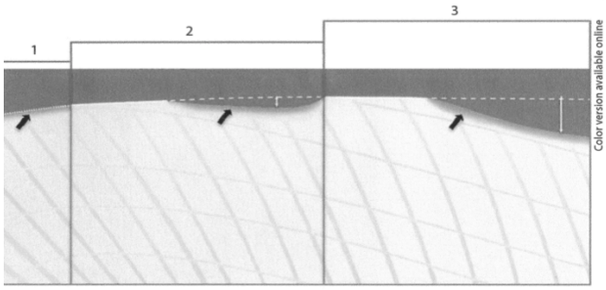

Figure 2. The different stages of dental erosion. On the left the softening of the enamel without tooth material loss. In the middle there is partial tooth material loss and softening of the underlying surface. On the right is significant tooth material loss. (Lussi et. al. 2011)

A study was done in which tooth samples were incubated in two different solutions, one at pH of 7 and the other at a pH of 4. Both showed a decrease in the minerals of calcium, zinc, and phosphate, with the solution at pH of 4 showing a greater decrease. This demineralization is characteristic of teeth in acidic conditions (Adhani et. al. 2015).

While erosion affects all teeth, the extent of erosion will differ for each type of tooth. A study was conducted using different types of teeth to compare the effects of acid and also to examine how the buccal/labial and lingual/palatal sides are affected (figure 3). The results showed that the lingual/palatal surface of the teeth showed greater loss due to acid dissolution than the buccal/labial side of the corresponding tooth. The palatal side of all maxillary teeth showed greater susceptibility than the lingual surface of the mandibular teeth. The teeth in the mandible showed great variation among teeth and based on surface, while the maxillary teeth showed similar dissolution for the palatal side and little variation among the buccal/labial sides. The difference can be due to the fact that the lingual/palatal sides of the teeth generally are observed have more wear due to being exposed to food for longer periods of time than the buccual/labial sides (Tucker et. al. 1998).

Acidity of Sports Drinks

The acidity of many sports drinks is much lower than the critical pH of 5.5, which has been shown to cause dental erosion.

Product	pH
Monster Assault	3.49
Red Bull	3.37
Gatorade Fruit Punch	3.27
Propel Mango	3.23
Gatorade Lemon-Lime	3.07
Full Throttle Energy Drink	2.94
Gatorade Cool Blue	2.92
5-Hour Energy	2.81
Powerade Red	2.77
Rockstar	2.53

Figure 3. The pH of many common sports drinks. (http://www.sheltondentistry.com/patient-information/ph-values-common-drinks/)

Erosion Caused by Sports Drinks

A study was performed to determine if there is an association between drinking sports drinks and dental erosion. Seven hundred and ninety-five patients filled out a self-administered questionnaire, which included their background, dietary habits, and frequency of tooth brushing. The patients then were checked for dental erosion, checking twenty surfaces in total among fourteen different teeth for each patient. A patient with three or more surfaces exhibiting erosion was considered to be affected. Of the people who had a low consumption of sports drinks, less than 0.24 liters/day, 26 percent were affected. Of those with moderate consumption, 0.25-0.75 liters/day, 41 percent were affected and of those with high consumption, more than 0.75 liters/day, 77 percent were affected. An athlete during training will drink at least 1.5 liters/day, which is much higher than those considered in the high consumption group for this study, and would therefore be highly susceptible to dental erosion due to these sports drinks. This study showed that there is an association between drinking sports drinks and dental erosion (Sovik et. al. 2015).

In a two-part experiment the erosive potential of 5 sports drinks was first checked in vitro for erosive potential and then the most erosive drink was tested to determine how it affected teeth in situ. The in vitro part took Gatorade, powder and liquid, Isotar, powder and liquid, and Isotar liquid and immersed six enamel samples for each solution for a total of four hours, checking the erosion every hour. The Gatorade liquid was the most erosive and therefore was used for the in situ part. In this experiment ten adults wore intraoral appliances that contained two teeth. The adults were then put on a drinking regimen of the Gatorade for ten days. Upon completion the samples were checked for erosion. The results showed marked erosion in three, mild erosion in two, and slight erosion for the remaining five. This showed how there is variability among individual susceptibility to enamel erosion. This variation can be due to many components including drinking habits, amount of saliva, and biological variation in tooth specimens. It also showed that individuals that are susceptible can have major erosion due to sports drinks (Hooper et. al. 2005).

In another experiment, Sting, a sports drink with citric acid with a pH between 2-3, was investigated for its effects on tooth enamel. Anterior teeth were submerged in Sting for five minutes every six hours for fifteen days. The results showed surface irregularities, pitting and structural loss of enamel. This experiment confirmed previous studies that beverages with "higher concentrations of citric acid have an aggressive effect on the enamel surface leading to its dissolution" (Kazmi et. al. 2016).

In another study, three sports drinks were tested for their erosive potential. The three drinks were Gatorade Citron, 5-Hour energy, and Powerade Cherry. Fourteen premolars were chosen for the study and were divided into groups for each drink. The teeth were exposed to the specified drink for four times for two minutes each over a one hour span every day for fourteen days. Compared to the control group held in artificial saliva, the Gatorade was the most erosive with an average loss of 10% of calcium ions and 8% of phosphorous ions. The Gatorade was followed by Powerade in erosive potential, having an average loss of

9% of calcium ions and 6% of phosphorous ions. The least erosive of the three drinks was 5-hour Energy, which showed an average loss of 5% of calcium ions and 3% of phosphorous ions. The results showed that each of the drinks caused erosion of the enamel and loss of calcium and phosphorous ions, with Gatorade showing the most erosive potential (Arnauteanu et. al. 2015).

In a study, three beverages and medicated cough syrup (Johnson and Johnson) were tested on teeth with and without restorations. The drinks were a carbonated drink (The Coca-Cola Complany), a non-carbonated drink (Parle Agro), high-energy sports drink (Red Bull). The sports drink had a pH of 3.26 but also compared to the other drinks had the highest neutralizable acidity. The specimens were in the high-energy sport drink for 350 hours over fourteen days, equaling fourteen years of normal beverage consumption. The teeth without restorations showed erosion and the restored teeth showed microleakage, due to the erosive effects of the sports drink. The drink contained citric acid which can "bind to calcium and phosphorous thereby promoting increased titratable acidity levels." The citric acid added to the drinks for flavoring agents leads to an increase in the sports drinks erosive potential. The microleakage caused by long term use of the sports drinks can eventually lead to restoration failure or secondary caries (Trivedi et. al. 2015).

Another problem for people who drink sports drinks is the hyposalvation that occurs during exercise. Due to the strenuous activity, an athlete can lose up to 1.5 liters of liquid from perspiration. This leads to a decrease in saliva resulting in xerostomia, dryness of the mouth. Saliva normally acts as a buffer and can neutralize the acidity of consumed liquids or foods. The saliva will also clear the liquids and foods quicker from the mouth thereby lessening the harmful effects of the acid. Even as acids cause ions to release from the teeth, the saliva can provide calcium and phosphorous to replenish the teeth. However, when there is less saliva in the mouth during an exercise the sports drinks' acidity will have an even greater effect than normal and cause more erosion than during rest. Also, because of this dryness in the mouth a person will usually drink more and therefore will have more acidic drink with less buffer (Noble et. al. 2011).

In the future drink companies are looking for ways to combat the erosive potential of sports drinks. By adding calcium, companies can see a pH adjustment which will reduce the erosive potential of the sports drinks, as it has been done for soft beverages (Arnauteanu et. al. 2015). This concept of adding calcium has also been tried with Ribena ToothKind. Compared to regular Ribena, the Ribena Toothkind showed significantly less enamel loss. (Milosevic 2004). If these results can be duplicated with sports drinks then their erosive potential will be decreased and be healthier for athletes.

Conclusion

Originally developed for professional athletes, sports drinks have become increasingly popular among the general population as well. However, studies indicate that the acidity levels found in sports drinks can cause dental erosion. Sports drinks have a pH well below the critical pH of 5.5 and therefore have harmful effects on teeth. The low pH of the drinks causes the minerals to diffuse out of the tooth and cause a loss in tooth volume. The acid also causes a loss in hardness, which can lead to further tooth damage. New ways of making these drinks may help mitigate the adverse negative effects of sports drinks on dental health.

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Possible Mechanisms That Protect the Fetus from Maternal Rejection

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Abstract

There is no other foreign tissue transplant that has such a strongly parasitic relationship with its human host as the fetus. Although the fetus contains paternal genes, is completely tolerized by its maternal host in almost all pregnancies. This presents an immunological paradox and has generated a lot of attention from leading researchers in the reproductive and immunology fields. This paper reviews the leading explanations for this paradox; that it is attributed to a detailed mechanism of the maternal and fetal immune system in which tryptophan suppresses T-cells from attacking specific paternal cells, while maintaining a strong immune response against other foreign antigens during pregnancy. Other opinions contribute fetal tolerization to the maternal immune systems strong bias of Th2 cells and a decrease of Th1 cells. Researchers suspect that women suffering from recurrent miscarriages are unable to tolerize their fetus, and consequently, their immune system attacks the fetus several weeks after implantation and aborts the pregnancy. Other medical implications include preeclampsia, which is attributed to immunological issues. Doctors are now trying to understand how these mechanisms work to provide treatment for women who cannot naturally tolerize their fetus, and for patients suffering from preeclampsia.

Introduction

The immune system is incredibly complex in its means of maintaining health and protecting the body against foreign invasion. It is a network of cells, tissues, and organs that work together to defend the body against "invaders" known as antigens. If the body recognizes a cell as being "non-self", it triggers an immune response against the antigen. The immune response includes different cells that the body stores in preparation for attack (Storey, Jordan, 2008).

All immune cells begin as immature stem cells in the bone marrow. They respond to cytokines (which are proteins involved with immune system cell communication) and other signals to develop into B cells, T cells, and phagocytes. B cells' main role is to secrete antibodies. When B-cells encounter an antigen, they create plasma cells for that antigen, which then secretes hundreds of identical antibodies that are specific to that antigen. Antibodies, also known as IG- immunoglobin, are highly specific proteins found in body fluids that identify antigens and connect to them with its specific shape and mark them for destruction. They neutralize the antigen and prevent it from attacking a host cell. Phagocytic cells then destroy the antigen (Storey, Jordan, 2008).

Another type of immune cell is called a T-cell. There are two different types of T cells; Helper T cells (CD4's) contribute by directing immune responses, and Killer T cells (CD8's) attack infected cells. The Major Histocompatibility Complex (MHC) are proteins on cell surfaces and code for the specific unique proteins that the cell contains. MHC protein bind to the surfaces of antigenic cells and help T-cells distinguish other cells as being self or nonself (Adar, et. al. 2015).

This leads to the concept of transplantation. If foreign tissue is implanted in the body, the T-cells will recognize the tissue's MHC as being nonself and will therefore attack it. A fetus has the distinction of being a tissue alloantigen in the mother's body. This is because it is impossible for a mother and child to be genetically identical since the fetus inherits a set of genes from each parent so the paternal genes are going to be considered foreign to the maternal immune system (Mellor, Munn, 2007). Furthermore, if the fetus is a boy, then they are certainly genetically different due to the presence of the Y chromosome in all males (Simpson, et. al. 1997). Consequently, one would assume that the maternal immune system would identify the fetus as being a foreign tissue and reject it. However, nature proves that this is not the case. Additionally, pregnant women do not seem to be more susceptible to infection. This proves that the maternal immune system is functioning and carrying out all other appropriate immune responses (Sacks, et. al. 1999). The purpose of this review is to explore this unusual relationship, and by surveying the recent literature, gain insight into the complexities of the immune system and explain this seemingly paradoxical relationship. The first to have raised the question of the maternal-fetal immunological paradox was Peter Medawar. His initial 1953 lecture and essay on this topic led to extensive research till this day. Retrospectively, scientists give him the distinction as the father of reproductive immunology. Upon doing pioneering research on skin graft rejection, Medawar wondered why a fetus is different than any other foreign transplant if the body clearly tolerizes its presence. He proposed three possible explanations; the mother and fetus have a physical anatomical separation, the antigenic immaturity of the fetus, and the immunological inertness of the mother (Billington, 2003).

Methods

Research was compiled from original journal articles, accessed through Touro's online library (www.tourolib.org) which has a subscription to the EBSCO and ProQuest databases. Key phrases such as maternal fetal immunology, rejection of fetus, and

tolerization of fetal allograft were searched to find relevant information. These were the basis for this comprehensive review of the topic.

Discussion

The first to have raised the question of the maternal-fetal immunological paradox was Peter Medawar. His initial 1953 lecture and essay on this topic led to extensive research till this day. Retrospectively, scientists give him the distinction as the father of reproductive immunology. Upon doing pioneering research on skin graft rejection, Medawar wondered why a fetus is different than any other foreign transplant if the body clearly tolerizes its presence. He proposed three possible explanations; the mother and fetus have a physical anatomical separation, the antigenic immaturity of the fetus, and the immunological inertness of the mother (Billington, 2003).

T cell Awareness of Fetal Presence

In response to Medawar's first hypothesis, research was done to test if the maternal immune system is aware of the fetus's presence. The trophoblast is a tissue layer that surrounds the embryonic cells. It supplies that embryo with nutrients, and its outer layer separates the fetal and maternal circulatory system throughout the entire pregnancy (Bonney, Matzinger, 1997). Researchers performed an experiment to discover if there are fetal MHC molecules at the extraembryonic tissues and if so, if the paternal genes are present in equal proportion to the maternally inherited genes. They implanted an embryo with paternal (foreign) and maternal (identical) MHC class 1 genes in a mouse and tracked them. On day 13 of gestation, fetal cells had low levels of MHC class 1 genes, yet equivocally high levels of both paternally and maternally derived genes were present at the interface of the extraembryonic and uterine tissues . Since the paternally derived genes are present in high numbers at the interface, it is unlikely that the paternal antigens are not accessible to the maternal immune cells. This study proves that the maternal tolerance cannot be contributed to a lack of paternal gene expression or to a lack of contact between the mother's immune system and fetal cells (Philpott, et. al. 1988).

Another study which strengthens this point was done on mice in which H-2K females were mated with H-2B males. For comparison purposes, they also mated these females to H-2K synergic mice and a third party, H-2s bearing mice. When tested mid-pregnancy, mice bearing H-2B conceptuses had reduced numbers of T cells with high expression of the clonotype and 6-9 times more clonotype positive cells that were missing CD4 and CD8 than the control mice. This reaction proves that the maternal T cells are exposed to and recognize the paternal alloantigen (Tafuri, et al. 1995).

They tested the mice further to determine if the T cell changes were still present after delivery of the fetus and found that the T cells were restored to the same levels as the control mice. However, that could just indicate that there is only tolerization when the paternal alloantigen is present in the body. To test this, after delivery, they introduced grafts of the H-2KB gene and the mice rejected it. They have also tried transplanting paternal grafts and have found that the mother's immune system only accepted it if the MHC peptide complexes on the graft were identical to that of the fetus. After the pregnancy, however, the maternal immune system did reject the graft. This study proved that pregnancy induces a transient state of tolerance for the paternally derived genes of the fetus (Tafuri, et. al. 1995).

These studies indicate that Medawar's first two hypotheses were wrong. The maternal tolerance is clearly not due to lack of exposure, and must be contributed to some mechanism which occurs during pregnancy that protects the fetus from rejection. As for his argument that the fetal antigenic cells are immature, we do see that fetal cells may lack high levels of MHC expression during gestation. However, he was assuming that the only exposure the maternal immune system has is from the fetal cellsf. As the above experiments proved, the trophoblast, which is definitely in contact with the mother's immune cells, had MHC genes at high levels from the beginning of gestation. Thus, it cannot be that the tolerization is due to antigenic immaturity.

Another study was done to test Medawar's third hypothesis which is that the mother is in an immunological tolerant state. They found that the T cells in the spleen and lymph node displayed characteristics that were typical of functionally unresponsive T cells and are not typical for antigen-experienced T cells (Zhou, Mellor, 1998). Researchers conclude from this data that maternal T cells are exposed to the paternal alloantigen's and this exposure somehow induces a tolerant state. Scientists are now focused on trying to figure out what exactly causes this tolerant state. However, it is clear that the mother's immune system is not suppressed, because it is responsive to all other antigens. Therefore, it would be inaccurate to describe the maternal immune system as being inert.

Indoleamine 2,3-dioxygenase (IDO) Mechanism

There is a mechanism discovered by Drs. Mellor and Munn which is currently the most well accepted explanation for this immunological contradiction. They attribute this tolerance to the fetus shutting off the mother's natural defenses. Their hypothesis is that the embryotic cells in the placenta, known as syncytiotrophoblasts, produce an enzyme called indoleamine 2,3-dioxygenase (IDO) which destroys an amino acid, tryptophan, that is necessary for the maternal T cells to protect the maternal body effectively (Munn, et. al. 1998).

In order to test this hypothesis, an experiment was done in which they had four groups of female mice. Two groups (A and B) were mated to mice that were genetically identical to them and two groups were mated to mice that were genetically different (C and D). Levels of IDO were first assessed in all groups. IDO transcription were present in all mice from 7.5 to 9.5 dpc (days post coitus). At later gestation times, such as on days 10.5 and 13.5, IDO was present in the placenta but not in the uterus or embryonic tissue. This data proves that IDO is only found in synctytiotrophoblasts and not in other tissue (Munn, et. al. 1998).

1-methyl-tryptophan, a pharmacologic agent that inhibits IDO enzyme activity was then inserted under the skin of mice from groups A and C. Groups B and D were used as a control. On 6.5 dpc, mice from all groups were carrying normal numbers of concepti and development was normal as well. However, from 7.5 to 8.5 dpc, the number of concepti in mice from group C (mice that were mated with allogenic males and treated with the IDO inhibitor) decreased significantly and there was hemorrhaging around the concepti that were left. At 8.5 and 9.5 dpc, all concepti showed signs of inflammation and deterioration. After 9.5 dpc, no concepti that were treated with the IDO inhibitor remained. To contrast, the concepti from groups A and B, fetuses whose parents were genetically identical, all survived and showed signs of normal development. This is despite group A having received treatment of the IDO inhibitor. Group D, which included the mice mated with synergetic partners, were not given IDO inhibitor and the pregnancy progressed normally and all concepti survived (Munn, et. al. 1998).

To test if a single paternal MHC class 1 difference will also lead to rejection, the scientists altered one gene and mated the mice. They found that the mice treated with IDO inhibitor all lost their concepti. The results of this experiments clearly indicate that IDO is an important factor in preventing the maternal immune system from rejecting the fetus (Munn, et. al. 1998).

A closer look at this data sheds some light on the mechanism that is likely to be responsible for protecting the fetus from rejection. Data showed that fetal rejection happened very early on in gestation, which does not give enough time for B cells to have produced the appropriate antibodies. This leaves it to T cells to have taken the active role in rejecting the fetus. Furthermore, it would be impossible to attribute the fetal loss to toxic effects of 1-methal-tryptophan, because the synergetic mice that did receive the inhibitor displayed no symptoms, indicating that it must have been an immunological effect of the inhibitor. This groundbreaking study indicates that it is the fetal allograft that protects itself from being rejected (Munn, et. al. 1998).

Many studies have since been done to test this hypothesis. Due to obvious ethical considerations, it is impossible to do complete experiments on humans, because of the rejection results that will likely occur. However, another research group tested for presence of the IDO enzyme in the human placenta. They first detected IDO at around week 14 and then levels increased rapidly and remained high throughout the pregnancy. When studying pregnancies with retarded intrauterine development, the IDO levels were significantly lower. This suggests that IDO enzyme is protecting the fetus from the maternal immune system (Sedlmayr, et. al. 2002).

Because tryptophan is an amino acid and cannot be synthesized by the body, the body's only source of it is from dietary intake. When placing golden hamsters on a high tryptophan diet, experimenters found that there was reduced embryonic survival and it influenced pregnancies adversely. This seems to link tryptophan levels with maternal rejection of the fetus (Meier, Wilson, 1983).

Another finding which seems to give credence to Mellor and Munns findings is the progressive decrease of tryptophan levels in serum from the beginning of human pregnancy until delivery. This shows a distinct inverse relationship between tryptophan levels and successful pregnancy in humans (Schröcksnadel, et. al. 1996).

In addition to the discovery of the role of IDO enzyme, further experiments indicate that there is another enzyme, Tryptophan 2,3-dioxygenase (TDO), which also contributes to tryptophan degrading activities. While establishing a time course, they have found that on days 9.5 to 12.5, IDO enzyme was at its peak expression. This phase coincides with the days of placental appearance and growth. In this experiment, IDO levels did decrease after this small phase, but tolerization lasted throughout the pregnancy which can indicate that once tolerance is established, it lasts despite continued tryptophan activity. However, on days 5.5 to 10.5, at the early stages of gestation, TDO enzyme was found at high levels. It is clear that during this early stage, IDO enzyme is not there, as 1-methal-tryptophan (an IDO inhibitor) was administered and did not inhibit the tryptophan degrading activities. This study suggests that early tryptophan inhibiting activities is due to TDO enzyme and during placental formation, IDO enzyme is responsible for inhibiting the T cell proliferation (Suzuki, et. al. 2001).

Th-2 Bias Mechanism

Many researchers contribute the tolerization to mechanisms other than the IDO enzyme.

Another mechanism that is now being researched is based on the link between successful pregnancy and a bias of Th2 cells. There are two types of helper T cells, Th1 and Th2. Th1 cell secretes pro inflammatory cytokines such as IFN-y and TNF-a. Th2 cells secrete anti-inflammatory cytokines such as IL-4,

IL-10, and IL-13. IL-4, which is produced by Th2 inhibits the growth of Th1 cells. IFN-y, which is produced by Th1 inhibits the growth of Th2 cells. Dr. Wegmann, hypothesized that in successful pregnancies, there is a strong bias of Th2 cells circulating at the maternal-fetal interphase. Because Th2 is anti-inflammatory, a significantly strong presence of Th2 cells will protect the fetus from th1 cells that cause inflammation and will allow fetal tolerization. In order to test this hypothesis, he injected pregnant mice with IFN-y and found that it resulted in pregnancy loss. This indicates that Th2 cells may promote maternal tolerance (Wegmann, et. al. 1993) .

Similar experiments done on both humans and mice have indicated this mechanism. Several studies showed that in normal human pregnancies there were increased levels of Th2 cell ration bias and at fetal loss there was a Th1 cell ratio bias (Ng, 2002). Furthermore, after examining samples from pregnant women, they found increased expression of IL-4 mRNA and decreased expression of IFN-y mRNA (Tranchot-Diallo, et. al. 1997).

A study of the th1/th2 cell levels in peripheral blood of women showed further links. Researchers examined the blood of women who have a history of recurrent miscarriages which is assumed to have been caused by maternal rejection. Within that group, they had a subgroup of women who were currently in middle of a healthy pregnancy and a subgroup of women who just had a miscarriage. They found that the women in the middle of a normal pregnancy had a strong bias of th2 compared to the women who just had a miscarriage and that the women who just miscarried had a strong bias of th1 cells (Makhseed, et. al. 2001). This implicates that th1/th2 cell ratio may play a significant role in the maternal tolerization of paternal alloantigens.

Nonconcurrent Theory

There have been suggestions that the fetal cells migrate to the maternal circulatory system: the lymph node, spleen, and thymus, where they will proliferate and then inactivate the potentially reactive T-cells. Several studies prove this to be the case with tolerized organ transplants. However, later research proved that it is highly unlikely that fetal cell migration can account for maternal tolerization. They found that, in fact, this scenario of fetal cell migration occurred in 1 out of 5 pregnant mice and at a level of 1-5 fetal cells per every 100,000 maternal cells. Because this event occurs so rarely, it doesn't seem rational to list fetal cell migration as a likely mechanism that protects the fetus from being rejected (Bonney, Matzinger, 1997).

Clinical Implications

Recurrent Spontaneous Abortions

As the experiments on mice indicated, fetal rejection resulted in the death of concepti. The death was caused by the maternal immune system's rejection of the placenta, which led to severe inflammation and hemorrhaging of the embryo, causing it to choke and die. This scenario can present in human pregnancy as well. Clinicians now wonder if the underlying explanation behind a lot of the miscarriages that occur is really the maternal immune system's rejection of the foreign paternal alloantigen (Mellor, Munn, 2000). Furthermore, if it is indeed the cause of recurrent miscarriages, then discovering the mechanism that induces maternal tolerance has clinical relevance as it is essential for proper treatment.

In order to test the IDO enzyme explanation for fetal tolerization, and to uncover if failure or dysfunction of this mechanism can lead to a miscarriage, researchers tested the cervical mucous, placental villi and decidua tissue. They surgically removed the tissue samples and mucus from women with recurrent miscarriages and divided the group into those that exhibited normal chromosomal groupings and those that did not. They compared the level of IDO enzyme presence to samples from women who had normal pregnancies and delivery. In samples of cervical mucous and decidua tissue they did not find a significant difference among levels of IDO in any group. However, when comparing villi from miscarried pregnancies of normal chromosome analysis and abnormal chromosome analysis, they found that the tissue from the normal chromosome group had significantly higher levels of IDO enzyme than tissue of the abnormal chromosome group. These results suggest that IDO enzyme dysfunction is linked to women suffering from recurrent miscarriages. Further research is necessary to test if IDO enzyme treatment can prevent women from miscarrying by preventing the maternal immune rejection that likely takes place (Obayashi, et. al. 2016).

Similarly, researchers who believe that a Th2 cell bias is responsible for maternal tolerance have studied the connection between helper T cells and recurrent miscarriages. In one experiment, they mated female mice who were deficient in IL-4 and IL-10 with male mice who were genetically different. They found that these mice experienced fetal loss. They treated some of these mice with an intraperitoneal injection of IL-10 and found that it protected the fetuses from resorption. (Sykes, et. al. 2012) Women who are suffering from recurrent spontaneous miscarriages are assumed to have a dysfunction in the immunological response to their fetus. This study implicates that by intervening and manipulating the levels of Th2 cells at the beginning of a pregnancy can protect these women from future miscarriages. Further research is being done on specific treatment methods.

Preeclampsia

Preeclampsia is a pregnancy complication characterized by high blood pressure. The high blood pressure seems to result from abnormal formation of blood vessels in the placenta. ET-1 is the peptide which causes vasoconstriction. Studies have found that

increased level of ET-1 in the plasma correlated with a bias of th1 cells. Upon further examination, this observation can indicate that if maternal tolerance mechanisms do not function normally, preeclampsia can occur.

When comparing women in middle of normal pregnancies to women with preeclampsia, they found that the ratio of th1/th2 was 7.6 in normal pregnancies and 11.6 in preeclampsia pregnancies. This shows that significant increases in levels of th1 are associated with preeclampsia (Kuwajima, et. al. 2001).

The th1/th2 cytokine imbalance that is found in preeclampsia can be explained since th1 cell is a proinflammatory cell and the vasoconstriction can be a result of inflammation of the placenta and umbilical cord (Vargas-Rojas, et. al. 2016).

Conclusion

The mystery of why a mother does not reject her fetus, as she would reject any other foreign object, is the focus of much research and academic debate. There is clear evidence to prove that the maternal immune system is in contact with the fetus and aware of its presence. The fetus is surely expressing paternal genes that are considered foreign to the immune system. Furthermore, the maternal immune system seems to otherwise function completely as normal, indicating that there is a specific mechanism which must protect the fetus. One suggested mechanism is that the fetus secretes IDO enzyme which inhibits a maternal amino acid, tryptophan, which supports the T cells of the immune system. This forces the maternal immune system to tolerize the fetus. Another explanation is that tolerization is due to th2 bias in t cell1/2 ratio. Th2 is an anti-inflammatory cell and can protect the embryotic tissue from a strong immune response. Details of these possible mechanisms has medical relevance for recurrent spontaneous abortions and preeclampsia because both conditions seem to be caused by immunological dysfunctions of the tolerization mechanism. Further research is being done to uncover other details of the mechanism and learn the entire explanation for this immunological paradox.

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Is Tooth Bleaching Really Safe?

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Abstract
The field of cosmetic dentistry emerged when people began to realize the importance of a good smile. Stains on teeth were no longer deemed acceptable with the advent of cheap and safe procedures like tooth bleaching. This new procedure replaced the older, more costly and invasive method of laminated veneers and crowns. The chemistry behind this bleaching occurs via unstable hydroxyl radicals and thus the question arose as to how safe this accepted procedure really is. The purpose of this paper is to analyze the negative ramifications of tooth bleaching and to determine if it's truly safe. The null hypothesis is that the procedure is innocuous and the status quo of cosmetic dentistry is appropriate. Data for this report was obtained from EBSCOhost, Google scholar and PubMed. Tooth sensitivity, oral mucosal and gingival irritation are among the most common side effects observed. More serious side effects like weakening of bond strength, leakage of restorations, cervical root resorption, bleachorexia and degradation of enamel matrix are all observed and are concluded to be serious issues. Though they are reported in the literature, carcinogenicity, mutagenicity and depletion of oral microbes are all determined to not be of any true concern. With a plethora of reasons to avoid tooth whitening, it's imperative that users be properly informed before commencing whitening. This will ensure that all possible measures to avoid these negative effects are indeed taken. Needless to say, the use of such toxic materials shouldn't be available OTC (over-the-counter) as they currently are. If a new and cheaper system is developed on the heels of the successful Pearl Brilliant White Ionic Teeth Whitening System, then bleaching will finally be a safe and universal procedure.

Introduction
In many social circles a good smile is considered indicative of a healthy lifestyle and a wholesome person. Anthropologists have shown that people with striking smiles are more successful and confident than their peers (Townsville Bulletin, 2005). It has been noted that the biggest indicator to a smile's importance is the recent surge in purchase of whitening products and the reading of articles pertaining to such goods (Kihn, 2007). By nature, most people have a low self-esteem and will do anything to gain confidence in themselves or others. This is the root cause for the recent popularity of whitening procedures being used by adults to restore their original white tooth shade.

Most people are born with the potential for untainted white teeth, yet when they reach their adult years this doesn't become the reality. What happened along the way to change this potential? The answer is that their teeth became stained in one of two manners, intrinsically or extrinsically. Extrinsic staining occurs when the enamel of the teeth is discolored by intensely colored pigments termed chromogens that possess ability to bind to its white, outer portion. Coffee, red wine, cola, and tea all have these chromogens and contribute to extrinsic staining. Smoking is another lead cause as the tobacco is composed of two different key components. Tar is naturally dark and promotes staining, while nicotine is colorless. However, when the nicotine is mixed with oxygen it becomes a yellowish surface staining material.

Intrinsic staining is an entirely different subject and is the result of a discoloration of the internal structure of the teeth, known as the dentin. Dentinogenesis imperfecta is a genetical disorder of tooth development which causes improper dentin formation and results in teeth that may take on a blue-gray or yellow-brown hue. Both deciduous and adult teeth are subject to this malady which may weaken teeth more than normal, making them prone to erode, break, and even become permanently lost. Although fluoride is necessary to help prevent decay, if taken too far and ingested in excessive quantities it can lead to fluorosis. Fluorosis will result in white streaks that appear on the teeth and can only be removed with dental measures like bleaching. Trauma to the teeth can either cause internal bleeding discoloration or alternatively lay down more dentin under the enamel layer. As a result of dentin being a darker shade than the enamel layer, the darkness shows through and gives off a darker appearance. Another source for internal staining is tetracycline staining. The minocycline binds to plasma proteins and becomes deposited into the collagen-rich connective tissues of the bone, teeth and pulp. It starts with a light yellow tinge and develops into stronger colors when it oxidizes upon exposure to light (Good, Hussey, 2003). Finally, the most prominent cause for the odd tone of teeth is the indefatigable age factor. As people age, their enamel wears thin and reveals the yellower dentin beneath it. All of these causes can lead people to seek change and investigate the subject of tooth whitening.

The tooth whitening that will be dealt with in this thesis is far more effective in removing extrinsic stains than intrinsic ones. Tooth whitening by definition means the reestablishment of the initial and natural color of the tooth, while tooth bleaching is going beyond that which is natural. However, being that the terms are used interchangeably in varying circumstances and throughout the literature, the same pattern will be followed here. Both whitening and bleaching will thus always be referring to the general color change, without discussing its earlier appearance. Furthermore, it must be pointed out by way of introduction that prior to bleaching, the way to change the color

of teeth was via laminated veneers or crowns. The invention of tooth bleaching was designed to be a more cost-effective and less invasive procedure.

The mechanism of the bleaching isn't fully understood but the principle concept is almost universally accepted. The active ingredient is either HP (hydrogen peroxide), or CP (carbamide peroxide) which breaks down into 33% HP and is thus a weaker version of the former. An oxygen species which can vary between perhydroxyl anion (H02-), hydroxyl radical (HO-) and various other radicals subsequently forms from the HP. The structure of the radical depends on the reaction conditions such as presence of transition metals, light, temperature and pH (Joiner, 2006). This radical reacts with the extracellular matrix portion, specifically the chromophores and pigments it contains, to degrade the stains formed on the surface of the teeth (Goldberg, et. al. 2010). The chromophores are the part of the molecule of a dye that is responsible for its color, while the chromogens are those substances that can be converted into a pigment or dye. In a broader sense, it can be said that HP or CP break stains into smaller pieces, making the color less concentrated and consequently the teeth brighter. In a more technical chemistry sense, it can be said that the reaction with HP or CP leads to the oxidation of carbon double bonds in organic chromogens. This in turn fragments the chromogens so that the power of their color is subdued or even eliminated (Carey, 2014).

A study aimed at determining the relative effectiveness of CP or HP tested six people, having three of them perform bleaching with 3.5% HP and the other three by using 10% CP. These two concentrations are equivalent, as the more complex CP molecule breaks down into 33% HP and 67% other materials. Comparing results by using the Vitapan Classical shade guide to test the percentage of color change, the two substances were deemed to be statistically similar. Canines and incisors both decreased a few shades despite the fact that the concentration of peroxide was on the lower side of the bleaching spectrum in both cases (Berga-Cabarello et. al. 2005). Although the sample size was small, it is sufficient as it is merely confirming that which was previously understood as common knowledge. It can be seen from this study that pure HP is a more potent whitener than CP, as CP requires three times the concentration for equal results. However, it has been postulated although not yet proven, that CP does have its own advantage over HP. Equivalent amounts of oxygen species are released in both HP and CP, but not in equivalent amounts of time. CP releases the oxygen species slower and is consequently more stable, yielding better long-term results. Due to a lack of experiments on this theory, further testing should be performed to substantiate this claim about the long-term results.

There are three methods of bleaching that effectively remove most extrinsic stains and some intrinsic ones as well. Whitening toothpaste isn't among these three as it is only effective for light surface stains. The first is the power bleaching method performed chairside by a dentist. It requires usage of a high concentration of bleach (usually between 30% and 38% HP) and is applied for a duration of 30 minutes to an hour. During such a procedure, the dentist creates a seal around the bleaching area to ensure that the highly-concentrated bleach doesn't end up being ingested or in contact with the gums and thus irritating them. A second method is the supervised take-home method that is also monitored by a dentist. First, the patient visits the office to create molds for the teeth that will be used for the treatment. Then the peroxide is given in a gel form to be placed in the trays and applied at the dentist's recommended concentration. This is usually between 10% and 20% CP (equivalent to 3.5% and 7% HP respectively) and involves duration of a few hours daily for a couple of weeks. Periodic complementary appointments at the dentist's office are recommended to ensure the success of the operation. A third method is the OTC (over-the-counter) bleaching method. The user can find these whitening strips or gels at their local pharmacy or even on the internet. The concentration of peroxide in OTC products is in the 5% to 7% HP range.

The advantage of the chairside power bleaching is that it can be done at a rapid speed, with high concentrations, expediting the bleaching process. Its higher price tag, requirement to visit an office for treatment and use of dangerous concentrations of powerful chemicals makes some people wary of its usage. OTC products are far cheaper and can be done at the user's convenience, however the lack of professional regulation makes some people hesitant to employ it. Furthermore, it can get messy and is less effective for strong stains, making some people question if it's worth the bother. The take-home method is moderately priced and is the intermediate between the fully regulated power bleaching and the more controversial OTC products.

A study was performed to compare the color change and rebound effect of power bleaching in comparison to take-home bleaching. Rebound effect is a measure of how quickly the results of the whitening fade and the initial discoloration returns. A split mouth design was used where twenty patients were randomly assigned chairside bleaching to either their mandibular or maxillary anterior teeth and then followed by take-home bleaching to the other. Excluded from this study as well as all other studies listed in this thesis were those with active caries, periodontal diseases, previous bleaching procedures and orthodontic treatments. Additionally, those with tetracycline-staining, fluorosis and those who habitually smoke were omitted. This ensured that the results would be a direct indicator to the effectiveness

of bleaching to standard patients with regular extrinsic stains, and not tainted by outside factors during the treatment time. Patients were evaluated by a single examiner, blinded to each patient's bleaching regimen, immediately after treatment and 2 weeks, 1 month, 3 months and 6 months later. The testing was done to measure for bleaching effect, rebound effect and color difference between post-treatment and unbleached teeth. The results showed that there was no significant difference between power bleaching and take-home whitening on any of the matters being evaluated except for rebound effect at the 6 month follow-up. While take-home whitening didn't have a distinguishable rebound effect at 6 months, the power bleaching did. This can be explained by the dehydration effect that power bleaching has on the teeth, which will interfere temporarily with the evaluation of color differences. It can be said that a lot of the color improvement associated with power bleaching is an illusion caused by this dehydration effect rather that an actual improvement in the tooth shade. Another explanation is the longevity of treatment. Take-home bleaching is continuous for two weeks which allows for bleaching demineralization to work together with natural remineralization and results in longer lasting effects. However, power bleaching is a one-time treatment and thus remineralization begins right away, resulting in faster regression of the whitening effect. Although regression occurs faster in the power bleaching, there is no overall statistical difference in the color comparison of post-treatment teeth and those untreated (Moghadam, et. al. 2013).

More recently, it has been suggested that the use of laser heating can enhance the effects of tooth whitening. Regular whitening without heating works because the peroxide releases hydroxyl radicals that diffuse into the outer enamel and break down the stains in a matter of hours. Lasers can heat the HP and expedite the chemical reaction that leads to radical formation, reducing bleaching time. However, despite the popularity of such heating devices, it really has no effect on the quality, durability or speed of the bleaching (Carey 2014). Although there are those who argue and that it does work faster, it is still more costly and has reportedly increased the subsequent tooth sensitivity. It can be concluded that the results of light-activated procedures are equal to those lacking such treatment and there is no real benefit to using such methods (Kihn, 2007).

A study was performed using third molars to test the effect of various concentrations of bleaching substances on the degree of whitening. Hydrogen peroxide of 5, 10, 15, 25 and 35% concentration was applied to test the degree of whitening each one would do in the same 3 x 10 minute sessions. Unsurprisingly the 35% HP was the most effective, while the 5% was the least effective in changing tooth shade. Furthermore, the 35% HP reached the maximum degree of whitening in just one session, showing that it's the fastest method. However, the 5% HP took a staggering 12 sessions to reach the same maximum shade. The expectation was that the relationship would be linear and that 5% HP would require only 7 sessions to equal its 35% HP counterpart. Instead, results showed that the number of sessions increase exponentially with lower concentration and that the relationship isn't merely linear. An explanation for this phenomenon is that tooth whitening is far more complex and involves numerous factors to attain the same results. Thus, the diffusion and reaction of the degraded components of the peroxide with chromogens may not work under expected patterns. Despite this strange peculiarity, once the maximum shade is reached, there is no difference between the higher and lower dosages of HP with regard to its longevity (Sulieman, et. al. 2004).

As is the case with most medications and procedures, tooth bleaching has its fair share of side effects. Is tooth bleaching really as safe as advertised? Are potential by-products transient or are their effects felt over the long haul? The null hypothesis prior to investigation of the subject is that there are no serious negative results to tooth bleaching, regardless of technique. The procedures are ADA approved and it's unlikely they would sanction the use of unsafe methods. Furthermore, the technique has been in use for many years and if it was really harmful, would undoubtedly have been banned by now.

Methods

Data was found using a variety of different internet sources. PubMed and EBSCOhost were very helpful in providing data. Additionally, Google Scholar was used as a powerful search engine.

Discussion

Dentin hypersensitivity (mostly thermal) is the cause for the aches and pains associated with tooth whitening. Increased sensitivity is the most common by-product and some degree has been reported in over 50% of patients (Jorgensen, Carroll, 2002). A survey reported that 78% of tooth bleachers experienced pains of some sort ensuing their bleaching regimen. The chemical process for whitening releases the dentinal plug that is thought to be protecting the region. With the plugs removed, the core of the tooth becomes exposed to things from which it is usually safe. A dentinal fluid flow occurs internally, as a result, and leads to the excitement of pulpal tissue and the consequent sensitivity.

There are two ways to counteract the sensitivity created by removal of the dentinal plug that accompanies tooth whitening. One method is to replace the dentinal plug by using dental sealants to cover the exposed root. Varnishes, bonding agents, and restorative materials are all viable ways to physically close the gap. Another related way is to commence the usage of fluorides which will decrease the permeability of the teeth. A different

approach is to cause depolarization of the nerve. Application of 5% potassium nitrate can cause a soothing effect on the nerve. It acts as a tranquilizer and slows the repolarization, which in turn eases the pain that is associated with the irritated nerve.

An experiment was conducted to compare the tooth sensitivity experienced in at-home bleaching with 10 and 20% CP vs. in-office power bleaching with 35 and 38% HP. Twenty-five patients for each of the four categories were gathered for this experiment. The at-home treatments were accompanied by the antidotes of potassium nitrate and fluoride to see if they would help. Tooth sensitivity was measured qualitatively, as each week the patients were asked if pain was absent, mild, moderate or severe. Thirteen percent withdrew with pain they deemed intolerable, showing that not all pain involved was so temporary and bearable. The results dictated that 43.2% of patients experienced pain, which fits well with Jorgensen and Carroll's results in 2003. The puzzling statistic was that a high volume of 71.4% of users of 20% CP experienced pain. This phenomenon was astonishing considering that only 15% of those power bleached with 38% HP experienced the uncomfortable sensation. As a whole, 9.5% from the take-home treatments in comparison with 4.3% of the in-office whiteners felt the sensation (Basting, et. al. 2012). This experiment indicates that there are other factors besides concentration that play a role in causing tooth sensitivity. Had it been solely based on concentration, then the chairside whiteners would have endured more sensitivity than their counterparts at home. Perhaps it can be concluded that the duration of the take-home bleaching made up for its lack in concentration. Another disappointing conclusion was the minimal effect that remedies like fluoride and potassium nitrate had on quelling the pain.

The split mouth design experiment also tested for tooth sensitivity and is important in regards to comparing sensitivity of at-home treatments vs. in-office power bleaching. At all the time intervals that color change was measured, tooth sensitivity was assessed by use of a visual analog scale. The results showed no significant difference between the two types of whitening treatments as each reported sensitivity in the 40-60% range. Using standard deviation this is deemed statistically insignificant and thus both were considered to be equally irritating (Moghadam, et. al. 2013).

Another adverse by-product of bleaching is the oral mucosa irritation that will occur if not applied properly. Oral mucosa is the mucous membrane that covers the entire inside of the mouth with the exclusion of the teeth. This protective membrane helps maintain oral health and is composed of strong keratin fibers which makes it resistant to injury. At a concentration in excess of 10%, HP is deemed to be corrosive to the mucous membrane and can cause burns and tissue damage. When power bleaching is performed it's imperative that there be something that holds back the highly potent peroxide from entering the oral cavity. Furthermore, patients shouldn't be numbed during such procedures, as they must be able to alert the practitioner in the event they feel a burning sensation (Li, 2011). However, a patient's perception of pain can't be relied upon and the dentist must constantly check the adequacy of the barrier that was constructed. It would be prudent to use some form of dyed substance to test how the barrier really is. If no die leaks through, it can then be considered safe enough to proceed with the whitening procedure.

A related issue is the gingival irritation that often occurs post-bleaching. Gums are a soft pinkish tissue that is composed of oral mucosa, and is vital in supporting, surrounding, and protecting the teeth. Issues with gums have been linked to cardiovascular and respiratory disease by some health professionals. Therefore, it is highly alarming that patients who have undergone whitening treatment have in certain cases developed gingivitis. This inflammation of the gums will lead to red and swollen gums that tend to bleed easily. The onset of gingivitis occurs because gums, like all oral mucosa, are subject to damage at concentrations exceeding 10% HP. The cause for gingivitis is an ill-fitting tray or a leaky and failing barrier during office whitening treatments. Although mucosal irritation is often temporary, gingivitis is a dangerous disease that must be taken into account when balancing the merits and dangers of whitening. With such dangers lurking, it's quite clear that such substances shouldn't be placed in the hands of minors or irresponsible people.

An interesting study of an innovative OTC bleaching tray system helps shed some light on the mucosal and gingival irritation that often accompanies whitening. Thirty-eight subjects were provided with the Pearl Brilliant White Ionic Teeth Whitening System which contains 9% HP and uses electrodes in the wall of trays to deliver an electrical current. The 4-15 milliamperes current activates the gel, causing it to diffuse through the enamel, and leads to the oxidation of pigments and chromophores that is standard in all whitening methods. The purpose of the electrical power is to speed up the formation of radicals and thus reduce application time of trays. The 38 patients applied the trays twice daily for five minutes and a mere five days. This contrasts with standard OTC strips which must be worn for excess of an hour per application and multiple weeks per cycle. Patients were checked after the first treatment and after five days for irritations, sensitivity and for effectiveness of the bleaching protocol. Results after the first treatment reported a mean improvement of 2.3 shades and only 20% discomfort, with two patients reporting slight burns of oral mucosa. After five days, only 15% of patients reported any discomfort and nobody had to stop treatment early. The average gingival score didn't have a significant change and there was no additional inflammation after application of

the gel. Only seven of 38 patients had any blanching of oral mucosa during any point of the treatment, and such side effects lasted just a few minutes and didn't require intervention. The results showed a sharp contrast between the electric powered tray system's 20% discomfort level and standard OTC whitening strips 50% sensitivity incidence (Ghalili, et. al. 2014)

The cause for the lower sensitivity and irritation prevalence may have been a result of the addition of potassium nitrate to the HP gel which slows repolarization of the nerve and lessens the pain. However, it was shown in an earlier experiment that such treatment doesn't necessarily work (Basting, et. al. 2012). A more likely explanation is the decrease in wearing time of trays and contact time of the peroxide gel. These results and accompanying explanation would fit well with the conclusion that was made earlier regarding that experiment, where it was stated that increase in duration of bleaching can lead to increased sensitivity. The usage of this novel OTC treatment is slowed by its heavy price tag of $200 per tray, but its prowess is important to note. If time can be reduced significantly in an affordable manner, then many of the main side effects of bleaching will disappear.

The most harmful effect that any substance can have is carcinogenicity, the ability to cause cancer, primarily by genotoxicity. Genotoxicity is the negative effect that harmful substances can have on the genome by causing mutations to the cell's DNA. The method for testing for genotoxicity is via a micronucleus test that quantitatively measures chromosomal damage by counting all cells that have inducted micronuclei into their cytoplasm after exposure to genotoxic agents. These micronuclei form when all or part of a chromosome isn't incorporated into a daughter cell during cell division. A high micronucleus count is indicative of severe chromosomal instability and genotoxic effects that pose a health risk. The DNA fragments will occur only in those cells that have completed one round of cell division after exposure to the genotoxic agent. The lack of incorporation of the micronuclei is due to a lack of centromeres that prevents the fragments from migrating to the spindle poles during late anaphase. The end result is that fragments are left behind and they form a secondary nucleus that is kept in the cell cytoplasm.

When compiling a list of the drawbacks to tooth bleaching, the potential correlation to genotoxicity and carcinogenicity must be thoroughly investigated. The theory had been proposed that HP may raise the carcinogenic effect, much like it does in experimental animals. However, it has also been argued that those artificial conditions are of no relevance to tooth bleaching, as they have much higher levels of HP than tooth bleaching does. A study was performed to find the genotoxic effect of 10 and 16% CP on bleached patients. Particularly concerning is the presence of reactive oxygen species in the peroxides that could damage proteins and cell nucleus. Thirty-seven patients were randomly divided into two concentration groups and given customized trays to wear for two hours daily for a duration of three weeks. Collections of gingival margin cells were taken at baseline, 15 days and 45 days by abrasion and then properly affixed to slides. One thousand cells were counted per slide and underwent a micronucleus assay. Comparing the results of the 10 and 16% CP there was no statistical difference between the rates of micronuclei formation at all three time periods. Most importantly, the rates were in fact on the lower end of the 0.3 to 1.7% range given in previous experiments (Bona

ssi, et. al. 2011). These results showed that when not applied for long periods of time or improperly consumed, the use of peroxides alone isn't cytotoxic. Hence it can be concluded that teeth bleaching doesn't pose a threat to human gingival epithelial cells (Almeida, et. al. 2015).

Another study corroborated the results of the previous experiment. Thirty smokers and thirty non-smokers were given 10% CP to be used three hours daily for three weeks. The goal of this single-blind trial was to compare the genotoxicity and efficacy of at-home whitening between smokers and non-smokers. The usage of a micronucleus assay is a good indication of cancer risk associated with genotoxicity, as most tumors in humans originate in the epithelium. The results indicated that bleaching didn't increase the frequency of micronuclei in the cytoplasm. The number of micronuclei was higher in smokers than non-smokers, but that was the case prior to baseline (the starting point used for comparisons). This is merely indicative of the genotoxic effect of habitually smoking, and is unrelated to its effect on bleaching. Smokers and non-smokers alike didn't have a significant increase in micronucleus formation after performing bleaching. Ten percent CP was thus proven to be safe when used at low concentrations for the three-week period that was required. The study did have limitations as it wasn't truly a blind examiner that was testing for genotoxicity. The smokers had a stench on their clothing and in their breath, giving away the identity of the group to which they belonged. Furthermore, the timing of the post-bleaching micronucleus assay wasn't optimal as it was given shortly after the whitening treatment. In contrast, the regeneration of the cells from gingival tissue takes approximately ten to twelve days. Thus, had the assay been performed two weeks later it's possible the results would have changed (de Geus, et. al. 2015). However, the limitations can be overlooked as the results are backed by other studies (Almeida, et. al. 2015).

A consensus opinion on the matter of genotoxicity and carcinogenicity is given in a recent review article. Direct contact with peroxide can cause genotoxicity in cultured cells and bacteria. However, when in the presence of catalase and other biological

enzymes, the effect is mitigated. The free radicals of the reactive oxygen species need to reach the DNA to inflict damage and the presence of metabolizing agents inhibits their ability to reach the target in vivo. Thus, while it is a threat to bacteria in a lab, in humans it isn't deemed a real threat. HP has a weak local carcinogenic potential and nothing more. The International Agency for Research of Cancer put HP in group three as unclassifiable in its carcinogenicity in humans. Most certainly, the mild dose of 10% CP found in many at-home trays is of no threat to those not already predisposed to oral cancer (Perchyonok and Grobler, 2015).

There was one case trial that did experience a higher rate of mutagenicity as a result of using tooth whitening in vivo on humans. Two different groups received different types of in-office bleaching. The first group used ZOOM2, a 25% HP that also features light activation. The second group received Opalescence BOOST, a 38% HP which had no light treatment. Cell samples were collected from both the upper lip lining and the gingival area, via swab technique. Each sample was collected before bleach application, immediately after and then 72 hours post-whitening. The collection immediately after bleaching was a control group, as there wasn't enough time for mutant cells to reproduce and appear in the results. The collection 72 hours after treatment was the experimental group, as that is ample time for reproduction of cells. Although there were only eleven members in each group, the design was to capture large effects and for this purpose Cohen's size conventions test determined that eleven was large enough.

Results showed slightly higher indicators of genotoxicity in BOOST, but both forms of bleaching caused a large increase in these markers. When comparing the control and experimental groups, BOOST saw a 157% increase in micronucleus presence while ZOOM2 experienced a 142% hike. These results contradict those of other studies, however, there are numerous explanations to reconcile the differing conclusions. The aforementioned experiments headed by both Almedia (2015) and de Geus (2015) used low concentrations of CP, while this experiment used high concentrations of the stronger HP. This may have led to the genotoxicity increase and wouldn't be indicative of issues in at-home bleaching. Furthermore, even power bleaching isn't necessarily problematic as there were flaws in this experiment. Five out of 22 patients had minor restorations which is usually grounds for exclusion, as they have a negative effect and increase the micronucleus count. Also, patient's lifestyles can't be controlled and while in other experiments they may have refrained from negative behaviors, this experiment may have been an exception. Alcohol usage and improper diet have been linked to an increase in micronucleus count. All of these explanations make this case seem as more of an aberration than a rule (Klaric, et. al 2013).

Tooth whitening can cause permanent damage to the enamel structure. In addition to the free radicals, CP produces urea which subsequently decomposes into CO2 and ammonia. This is key in the bleaching process as the urea degrades the organic matrix in the enamel. Hydrogen bonds in matrix proteins are dissociated by the urea and ammonium ions. These empty spaces caused by the degrading of matrix proteins make possible for penetration of the free radicals to enamel and even dentine layers. However, whatever breakdown the urea creates is in fact real and permanent damage to the enamel and is one of the more serious issues of tooth whitening (Elfallah and Swain, 2013).

While enamel erosion is a serious issue, it has become well publicized that remineralization agents are a viable method for restoring tooth structure. An experiment was conducted to test enamel erosion generated by two different high concentration HP whiteners. Opalescence BOOST was used as a substance that is chemically active, while Mirawhite is a 30% HP substance that is activated by a diode laser. The experiment also tested four different remineralization agents to see which would be most effective in restoring initial tooth structure. Twenty-five molars for each whitening type were each subdivided into five groups, which featured one control group and four different remineralization experimental groups. The exact statistical measures for erosion and remineralization are unimportant, but the generalizations were quite startling. SEM/3D-SEM-micrographs revealed that both types of bleaching caused emphasized perikymata, which are the pits surrounding the long prisms of tooth enamel. These emphasized perikymata as well as the loss of interprizmatic substance both clearly indicated enamel erosion. These negative signs were even exacerbated in the teeth that were activated by the diode laser. Remineralization occurred in all four experimental groups, with calcium phosphate proving to be the best at covering the surface of the enamel. SEM/EDX-semiquantitative analysis showed that certain crucial elements were reduced from the tooth structure as a result of the bleaching procedure. Sodium and magnesium were most prolifically lost in the non-laser bleaching, while calcium and phosphorus were the hardest hit by the laser bleaching (Coceska, et. al. 2016).

Although remineralization agents can help repair the erosive effects of bleaching, this only works if patients are properly informed to commence application upon the onset of whitening treatment. However, users of OTC products are generally not properly informed and also further their plight by not reading the instructions. Thus, even when side effects are indicated in the user's manual, most consumers remain oblivious to the need for these remineralization agents. The loss of enamel causes a decrease in insulation from potential painful temperature and dangerous chemicals and can also lead to decay. Furthermore, enamel erosion makes the tooth more prone to chipping. Once

the enamel is lost it has no living cells to repair itself. All damage is permanent and costly alternative treatments such as bonding are now required.

The leakage of restorative materials ensuing tooth whitening is another major by-product of the procedure. Restorative materials have been used for many years to fill caries, repair damage due to trauma and much more. Originally amalgam was the primary restorative material, until a recent surge in the use of composite resin material. Issues arise when there is a leakage of mercury ions from amalgam upon the initiation of bleaching. Mercury ions can be toxic and lead to numerous diseases when the threshold concentration is reached.

The amalgam's natural release is a redox (oxidation-reduction) reaction in which the mercury metal reacts with non-metallic elements to produce chemical compounds (von Fraunhofer and Staheli, 1972). This same reaction would take place in vitro, as the redox reaction takes place at the amalgam/bleach interface resulting in the deposits. An experiment was thus conducted to investigate how much of a role both concentration and time of treatment have on the release of mercury. Tytin amalgam contains 42.5% mercury and is a typical dental restorative material. Sixty-five discs of tytin amalgam were prepared and divided into thirteen groups of five for the experiment. Four groups of discs were each treated with 0%, 3.6% and 6% HP. The various groups had varying times of exposure to HP of 1, 8, 48 and 156 hours respectively. The 0% HP groups were the control groups and contained saliva and other biological enzymes in place of the peroxide. The 3.6% HP groups represented the classical at-home concentration and the 6% HP represented a stronger version of these groups. The various times made this into a double experiment that charted both concentration and time of exposure against amount of mercury ion leakage. The thirteenth group was treated with 30% HP for one hour and was an imitation of in-office power bleaching. Each disc was measured five times for amount of mercury ion release and each group had five discs to ensure the accuracy of the measurements.

The results showed a greater release of mercury ions as the concentration of bleach was increased. Time caused increased release until the eight hour mark, at which point its effect plateaued. This showed that concentration was of greater effect than time and thus power bleachers should be cautious before starting whitening. However, the small amounts of mercury released don't produce effects on humans, as the quantities are well below the acceptable daily intake of forty micrograms. The maximum sum released by any of the discs was 1.125 micrograms and thus would require 36 teeth with restorations to pose any threat. Despite its relative safety, it's still not healthy to have any amount of harmful chemicals in the body and thus the release of amalgam is a side effect that must be taken into consideration when considering bleaching. In fact, this danger has caused Norway to ban amalgam restorations now that safer alternatives are available (Al-Salehi, 2009).

Scientists hypothesized that upon the onset of bleaching, an additional consequence would result from the redox reaction that occurs at the dentin. They feared bond strength at the dentin/resin interface would be adversely affected. To confirm this suspicion, they performed an experiment to test all facets of bond strength after application of varying concentrations of bleach to teeth. For the shear bond strength test, forty slabs of intracoronary dentin were obtained and split into four groups. One was a control group that was treated with artificial saliva that had no HP concentration. The second group was 20% HP and also had sodium perborate (a bleaching agent), a third group was comprised of 37% CP and a fourth group of 38% HP. Manufacturer protocol was performed for all bleaching regimens and a seven day waiting period ensued as a means to offer appropriate time for the residual bleach to leave the dentin. These teeth then received a shear bond strength test in a universal testing machine. Failure modes for the test were observed via microscope. Next, a flexural/fracture strength of dentin test was done on forty dentin bars from the cervical area of the buccal portion of roots. These forty bars were divided into the same four groups, underwent the same treatments and then received a three point test carried out by a universal testing machine. Finally, an SEM analysis of dentin surface and adhesive interface was prepared with five hemi-sections of lingual surface of crowns, for both the dentin surface and adhesive interface.

The results showed that shear bond strength of the control group was nearly double to that of the experimental groups. The unbleached teeth had mixed failure modes of both cohesive and adhesive failures, while the bleached groups had predominantly adhesive failures. Flexural strength was statistically significantly higher for the unbleached group than the experimental groups. The 38% HP was the weakest of all groups, although it was statistically similar to the 20% HP coupled with sodium perborate. Lastly, unbleached teeth had SEM analysis that showed dentin surface covered with its smear layer, the two middle groups had some areas with fissures and the 38% HP sample had cracks all over the specimens. Analyzing dentin/material interface there was a continuous interface in the unbleached group, and progressively more discontinuity areas with the higher concentration bleached groups.

The explanation for the weaker shear bond strength in bleached groups, is that hydroxyl radicals penetrate into dentin and break down connective tissue, such as collagen and hyaluronic acid. This in turn increases dentin permeability, reduces hardness and

leads to the decrease in shear bond strength. The oxygen inhibits the entrance of the resin/material into dentinal tubules and prevents their polymerization. Even after seven days, residual oxygen remains and causes adhesive failure. Hence, the analysis of failure modes indicated more adhesive failure for bleached teeth, while unbleached teeth had less adhesion failure modes and instead more cohesion failure modes. This furthers the notion that hydroxyl radicals formed from bleaching products interferes with the bonding of restorative materials. This may also be a secondary reason for leakage of amalgam restorations, as the failure to properly bind at adhesive interface causes the subsequent leakage. The SEM results were consistent with those of the shear bond strength test, as those with the highest HP concentration had more cracks in the dentin surface than those with lower HP. Finally, the flexural strength test confirmed the scientist's fears, as those with higher HP had less strength and would thus fracture faster in-vivo. All of these test results can be explained with the common theme, that the hydroxyl radicals ruin the structure of teeth while also reducing the ability of the resin to properly bond to the dentin (Vieira, et. al. 2012).

There have been numerous mechanisms proposed as ways to reduce and prevent the microleakage of composite resin restorations. This microleakage is particularly common when bleaching is done just prior to or soon after installation of the restoration. The bleach leaves behind residual peroxide that doesn't allow for proper polymerization of the resin to the remaining portion of the natural tooth. A test was done to compare various suggested means of mitigating the microleakage effect. Sixty intact premolars were split into six groups for the purpose of this trial. Group one was the control group, as the teeth were merely treated with saliva instead of the 10% CP applied to other groups. There has been a theory that allowing a three week time delay between bleaching and bonding would be ample time to allow residual peroxide to dissipate out of the teeth (Bittencourt, et. al 2010). Thus, group three was treated with 10% CP followed by a three week delay before installation of fillings. Group two provided the proper contrast to group three, as it was treated with 10% CP and didn't have the deferral of restorations found in group three. Group four had sodium ascorbate applied in between bleaching and the filling of caries. This chemical is an antioxidant and was seen as a faster alternative to the potentially equally effective but highly time consuming delay period. A recent study suggested that addition of surfactant (0.2% Tween 80) would enhance sodium ascorbate's ability to prevent microleakage (Moosavi, et. al 2010). Thus, group five presented sodium ascorbate coupled with surfactant treatment between bleaching and restorations. Finally, group six was treated with catalase instead of the antioxidant and surfactant, following a report that catalase removes residual HP from the surrounding area after bleaching (Rotstein, 1993). Microleakage was measured semi-quantitatively by the accepted criteria of the depth of dye penetration at the interface between restoration and cavity wall.

Data from the trial indicated a significant difference in amount of microleakage between the unbleached group one and bleached groups two through six. Furthermore, group two had the greatest microleakage as it had no preventive measures implemented preceding addition of composite resin. Groups five and six, although significantly greater in microleakage than group one, was significantly less than group two. It is thus evident that sodium ascorbate in conjunction with surfactant and catalase by itself are a sufficient method of reducing (but not completely terminating) composite resin microleakage. Groups three and four were statistically similar to group two, showing they were relatively ineffective at preventing microleakage (Han, et. al. 2014).

Extending the theory that explained the results found in this microleakage experiment, one can opine that application of catalase can also help cure the woes of the weakening of bond strength caused by whitening. This in fact concurs with a previously performed experiment which also concluded that pretreatment of bleached surfaces with catalase prior to bonding improves composite-enamel bond strength (Kum, et. al. 2004). On the surface this seems very reasonable, as one of the causes for both microleakage of restorations and weakening of bond strength, is the oxidative materials left behind after bleaching which prevents polymerization of installed materials to the natural tooth. If catalase can serve as a deterrent to microleakage it should then follow that it should relieve the stress on bond strength that the same residual harmful materials cause. However, catalase wouldn't be of any help for other side effects mentioned earlier in this report, as those aren't a result of the residual oxidative materials that the bleach leaves behind.

Cervical root resorption (reabsorption) is a naturally occurring process in primary teeth, as the deciduous teeth are uprooted to make way for the permanent teeth. This process is caused by the osteoclast differentiation that results from the pressure applied by the newly emerging teeth. However, as a result of trauma or excessive pressure of various orthodontic treatments, it's possible for a pathogenic resorption/breakdown of permanent teeth to occur. Such a condition can ruin a tooth if not properly treated. The problem is that this phenomenon is painless and unless detected via x-ray will go undiscovered until after carious lesions have taken hold in the external tooth. Bleaching is one of the orthodontic treatments that is a root cause for resorption due to the pressure associated with it. The disease is more commonly observed in those using HP bleaching than those using sodium perborate alone (Fearon, 2007). Sodium perborate is a milder procedure with less side effects, but shorter sustained

results. Thus, the intensity of the bleaching regimen clearly has a direct effect on likelihood of cervical root resorption. Use of heating devices is another catalyst for this malady. This is a logical consequence, as the heat generates hydroxyl radicals from the HP which are highly reactive and subsequently break down connective tissue found in teeth. Together, high concentration HP and heat can be a lethal combination for those trying to preserve their teeth. Another explanation why bleaching causes root resorption, is that the acidic environment that the bleaching procedure supplies enhances the disease (Dhillon, et. al. 2011). The diffusion of hydrogen ions from the bleach makes the region more acidic and creates an environment that is ripe for bone resorption and osteoclastic activity. The proof to this theory is that osteoclastic activity is strongest in 35% HP (3.7 pH), intermediate in 35% CP (6.5 pH), and weakest in sodium perborate (pH 9.9), a basic substance (Dhillon, et. al. 2007)

Additionally, it has been suggested that the acidic environment that bleaching creates can adversely affect the beneficial microbes that regularly grow in the oral cavity. It's important to have these essential microorganisms so that when adverse, exogenous viruses invade they are outnumbered and combatted by the symbiotic microbes. The harsh, acidic conditions could prove to be too much for the microbes to handle and thus diminish these protective organisms. Such a chain of events would leave whitening users with a greater risk for microbial disease. Four groups of eight were generated to test the effect various treatments and combinations of treatments would have on the overall concentration of microbes in saliva. The results would be a direct indication of the overall concentration in the oral cavity. The first group was treated with in-office CP 37% and at-home CP 10%. The second group received the in-office CP 37% and an at-home placebo, the third group an at-home 10% CP and an in-office placebo, and the final group a double placebo. All patients were given uniform brushes and dentifrices and inasmuch as possible were left under similar conditions. The in-office bleach was conducted in three sessions of one hour and the at-home whitening was three weeks in duration. Saliva was taken at baseline, right after application of bleach, twelve hours later and repeated each week during treatment. The results were placed on various culture media, but all results showed no significant difference between microbial levels at various periods. Thus, it was concluded that the bleaching of teeth has no effect as an antimicrobial agent (Franz-Montan, et. al. 2009).

The final major side effect of bleaching teeth is the potential to develop an addiction to the bleach. Such a disease is known as bleachorexia and those afflicted are dubbed bleachorexics. Much like anorexics who are convinced they aren't skinny enough, bleachorexics are convinced that their teeth aren't white enough. Instead of accomplishing a nice hue, these fanatics whiten to the point where teeth reach a translucent blue or grey appearance. This looks unnatural, especially when contrasted by a person who may have a darker skin tone. Bleachorexia can lead patients to turn an eight week regimen into a full-year program. These tooth whitening junkies present an added health risk with gum, tooth or even throat problems from repeated exposure (Bee, 2006). The relatively recent increase of bleachorexics is due to the prevalence of OTC methods which allows patients to take whitening into their own hands. The enamel becomes permanently damaged, root canal problems arise and free radicals damage cells and pulp in teeth due to the over-indulgence of bleach. Gums may recede, teeth become weaker and all other aforementioned side effects become amplified by the excessive use. Psychological intervention may be required to relieve patients of their plight.

Prior to drawing any conclusions, it's important to examine the long-term effectiveness of tooth whitening. A study was conducted to test for any difference in rebound effect at the two-year mark vs. baseline and the one-week mark vs. baseline. This was done for both at-home bleaching and in-office bleaching, to test which one has more sustainable results over the long haul. The general perception among clinicians has been that the at-home bleaching lasts longer than the in-office bleaching. Results in the split-mouth design experiment corroborated this general view (Moghadam, et. al. 2013). For this experiment thirty patients were given power bleaching for two sessions of 45 minutes during a one-week span. Another thirty patients were given at-home bleaching kits of 16% CP, to be applied for six hours per night for four weeks. Color change was detected using the Vita Lumina shade guide and was measured at baseline, one week later and two years subsequently. Results for at-home whitening showed a mean increase of six shade guide units for both one week and two years successive to bleaching. Rebound effect was 0.25 shade guide units over two years and this was deemed statistically insignificant. The in-office bleaching indicated a 5.5 shade guide unit improvement for both one week and two years after whitening. Rebound effect was 0.30 shade guide units and this too is considered statistically insignificant. Contrary to the common perception, the longevity of results for in-office bleaching was up to par with the take-home bleaching's durability (Tay, et. al. 2012).

The difference between the results of the in-office bleaching's six-month instability in Moghadam's experiment and the two-year durability in Tay's experiment is a simple distinction. In Moghadam's experiment only one session of 45 minutes was given to chairside bleaching patients. In Tay's experiment two such sessions were administered on each patient. According to some experts, it is only after the second bleaching of in-office treatments that tooth color does

change significantly (Al-Shethri, et. al, 2003). Thus, to equal the long-term stability of at-home bleaching, two sessions of chairside bleaching are required.

Conclusion

Although tooth whitening doesn't create a higher probability of carcinogenicity or genotoxicity, it is far from innocuous. Almost 50% of patients experience some form of tooth sensitivity for the first month of treatment. Oral mucosal and gingival irritation are very common as a result of ill-fitting trays and the subsequent leakage of peroxides. Tooth integrity is affected as a result of urea degrading the enamel matrix, and hydrogen bonds in matrix proteins becoming dissociated by the urea and ammonium ions. Due to the redox reaction, there is often an increase in the leakage of restorations, most notably amalgam. Weakening of bond strength is a direct result of the oxygen molecules causing a failure of resin to properly bond to dentin. Cervical root resorption is more likely to occur following the orthodontic treatment associated with bleaching. Finally, there is even a psychological issue named bleachorexia that is caused by a whitening obsession. With this excessive list of side effects it is clear that such dangerous substances must be regulated to some degree and not available over-the-counter as they currently are. It is a travesty that such harmful materials are accessible to minors and are not exclusively in adult's hands.

Various methods have been mentioned throughout the course of this work to help mitigate a number of the by-products of bleaching. While long-term rebound effect is unchanged by the form of bleaching (when power bleaching is done twice), the form of peroxide does matter. CP is said to yield longer-lasting results due to its slower and more stable release of oxygen species. Laser heating doesn't speed up the reaction rate and only serves to increase tooth sensitivity and speed up enamel erosion. Fluoride and potassium nitrate don't mitigate tooth sensitivity but decreasing the application time of bleach certainly can. In fact, usage of a novel electric powered bleach showed that decreasing wearing time can also cause a decrease in gingival and oral mucosal irritations. The best remineralization agent is calcium phosphate and should be taken in conjunction with whitening. Catalase helps remove residual oxygen species and should also be taken while commencing bleaching. The catalase should help relieve some of the woes of weakened bond strength and the leakage of amalgam restorations. Cervical root resorption can be diminished when a lower concentration of peroxide is used and laser treatment is avoided. Thus, the properly informed whitening patient can be shielded from some of the by-products of bleaching if he/she is proactive in treating them. However, the issues arise for patients who are not properly informed or are negligent in providing the proper care for their teeth.

It is clear from the abundance of research provided in this report, that while cheaper, whitening isn't a safe alternative to laminated veneers or crowns. Thus, those who can afford to do so, should choose the more conventional route when looking to make cosmetic repairs on teeth. The future of whitening seems to lie in the ability of companies to create a cheaper system that allows for shorter exposure time to peroxide. The Pearl Brilliant White Ionic Teeth Whitening System which contains 9% HP and uses electrodes in the wall of trays to deliver an electrical current, is definitely a positive start for making this a reality. Ultimately this system needs to be tested more to confirm that it really is effective, while decreasing the side effects. Furthermore, the hefty price tag doesn't allow this brilliant technology to gain enough popularity. A cheaper alternative must be created to allow all members of the populace to have access to this newest advance in the field. Only when this comes to fruition will whitening truly be a safe and cheaper alternative to laminated veneers and crowns.

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An Analysis on Whether or not Baldness can be Reversed

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Abstract

Many men experiencing hair loss often wonder: is there a cure? Can I get my hair regrown? This thesis addresses these very issues, the anatomy of the pilosebaceous hair, the hair growth cycle, and the suggested causes of male male pattern hair loss are examined. Finally, the Various drugs that have been suggested to reduce hair loss and even cause hair regrowth are reviewed. After examining the various treatments, it can be concluded with reasonable certainty that hair loss can be halted and often reversed by using FDA approved drugs finasteride and minoxidil. Other drugs such as ketoconazole and dutasteride have also demonstrated effectiveness in treating hair loss, however no FDA approval has yet to be issued, either due to lack of evidence demonstrating their efficacy, or due to concerns of negative side effects. The major disadvantage of using hair loss drugs is that one must continuously use the drug to maintain its benefits. It is therefore plausible to conclude that as of yet hair loss cannot be cured, rather its progression can be prevented as long as one uses the drug.

Introduction

Hair loss, known medically as alopecia, is a very common phenomenon, affecting both males and females. Demographically, it effects about half of males and a quarter of females by the age of 50 (Vary , 2015) This makes it very likely we will be affected by hair loss during our lifetime. Hair loss can manifest itself in various forms, the most common being male pattern baldness defined as the progressive loss of hair beginning in some cases at puberty, and proceeding throughout adulthood (Berman, 2015). Another form of hair loss is alopecia areata, an autoimmune condition where the body mistakenly attacks healthy hair follicles. Alopecia areata effects men, women and children with hair loss occurring in the form of patches on the scalp, and in extreme forms, a complete loss of hair (subtype known as alopecia totalis) (Moskowitz , 2014). True, hair loss is not a disease in a sense that it effects our physical wellbeing, however, it is a condition which may cause severe psychological distress, particularly anxiety and depression upon the affected individuals (Hunt, McHale, 2005). These psychological effects, combined with the widespread prevalence of hair loss make it imperative for us to find a cure. In this paper, the primary focus will be on exploring whether or not hair loss can be prevented, and if hair lost hair can be regrown. Since hair loss due to hormonal causes is the most prevalent and studied form, it will be the focus of this paper.

Anatomy of the Hair Follicle

To get a better understanding of the mechanisms responsible for hair loss, it is vital we discuss how hair growth occurs in a healthy individual. In mammals, formation of the hair follicle takes place during fetal skin development. Cells which form the hair follicle, sebaceous and apocrine glands (responsible for producing oils and sweat) are all derived from ectodermal stem cells. In contrast, cells derived from mesodermal stem cells will develop into the follicular dermal papilla and the connective tissue sheath, while the neural crest derived melanocyte progenitors produce the pigmentary cells, which are responsible for the coloring of hair (Fuchs, 2008).

Although hair follicles vary in shape and size, they all have the same basic structure. The base of the follicle known as the papilla, is primarily composed of connective tissue and a capillary loop which supplies nutrients to the hair follicle. Cell division at the papilla is very rare (Pawilna, Ross , & Kaye, 2003).

Superficial to the papilla, lies the hair matrix, the site of hair formation. In mammals the matrix contains trichocytes, the cells responsible for hair production. These epithelial cells produce modified keratin proteins, which contain ample amounts of the amino acid cysteine. Cysteine has a reactive sulfhydryl group which creates both inter and intra-chain linkage within a protein structure, thereby giving hair high tensile strength, and flexibility (Langbein & Schweizer, 2005). Also, contained within the matrix are scattered melanocytes.

Just superficial to the matrix, lies the root sheath. The root sheath is further subdivided into the inner and outer root sheath. The inner sheath contains three different layers, a cuticle layer, Huxley, and Henle's layer. The outer layer contains the bulge, a stem cell rich area which supplies the entire follicle with new stem cells. These stem cells are vital in the healing process of an epidermal wound. Also, contained in the outer sheath is the sebaceous gland, responsible for hair lubrication. In the uppermost region of the outer sheath are the attachment sites of the arrector pili, smooth muscles that serve to help the hair maintain a vertical position (Ma & Yang, 2004). The entire unit consisting of the hair, hair follicle, pili muscles and sebaceous gland is referred to as the pilosebaceous unit (PSU).

Hair Growth Cycle

There are three phases of hair growth, the anagen, catagen, and telogen. The anagen phase is what is known as the growth phase. During this stage the cells in the root divide rapidly. After dividing, the cells produce a new hair that pushes the old hair out of the shaft. At this time, the hair grows approximately 1cm every 28 days. The anagen phase is active for about 2-6 years.

Individuals who have trouble growing their hair to a proper length might have a short anagen phase, whereby their hair falls out to a renewed anagen phase. In contrast, individuals who have the ability to grow their hair exceedingly long are likely have a growth phase lasting very long. It should be noted that auxiliary hair such as that of the eyebrows, eyelashes and arms have a very short growth phase, lasting 30-40 days. This is why we don't have eyelashes that are more than a few millimeters in length.

The catagen phase is a transitional stage, which lasts from two to three weeks. At any given time about 3% of all hair are in this stage. During the catagen stage, hair growth stops, and the outer root sheath shrinks due to its detachment from the nutrient rich capillaries. At this point, a hard, club begins to form at the base of the hair, which is composed of hard keratinized tissue. This club holds the hair in place for as long as three months.

The telogen phase is the resting phase and usually last for about 100 days on the scalp and longer for the hair of the eyebrow, eyelash, arm and leg. During this phase the hair follicle is completely inactive, and the club becomes more solidified. If one pulls out a hair at this stage, a hard, dry and solid root will be visible. In a normal individual, about 25-100 telogen hairs shed each day (Elzouki, et al. ,2012). People with androgenetic alopecia don't have regrowth occurring at the same rate. Typically, the hair loss begins above the temples and vertex of the scalp, and as it progresses, a rim of hair remains at the side and the back of the scalp.

Causes of Male Pattern Baldness

Androgens and their role in hair loss

Androgens (the hormones responsible for the characteristic male appearance) play an important role in some, but not all hair growth. During puberty, the body produces significantly greater amounts of androgens to stimulate male development. One of the more noticeable effects androgens have is on the pilosebaceous units in the pubis, axilla (armpit), and lower face. In these regions, the hair goes from a fine, straight and almost colorless appearance, to a darker, thicker and curlier appearance. Additionally, in the areas of the upper face and trunk, the pilosebaceous units respond to these same androgens, by drastically increasing the size of the sebaceous gland, thereby increasing the amounts of oils they produce (Alonso & Rosenfeld, 2003).

In the male body, the major bioavailable form of androgens is testosterone. Testosterone can also be converted to a similar compound know as dihydrotestosterone (DHT) by the enzyme 5α–reductase, which reduces the 4,5 double bond. DHT has a significantly greater binding affinity and lower dissociation constant with the androgen receptor when compared to testosterone, hence DHT is a lot more potent. In men, approximately 5% of all testosterone molecules get converted by 5α–reductase into DHT (French, et al., 1990).

The importance of DHT in males is clearly demonstrated in individuals who have a deficiency in the 5α–reductase. Such individuals display phenotypical pseudohermaphroditism, a condition where the male genitalia and prostate are underdeveloped, even though they have a genetic makeup of a male (Imperato-McGinleyet al., 1979).

The androgen receptor is a 110kD protein with a ligand bonding domain, a DNA binding domain and two activation function regions that confer transcriptional regulatory activity. When a ligand binds to the androgen receptor in the cytoplasm, it exposes the nuclear localization signal. This allows it to dimerize with another androgen receptor and then be transported to the nucleolus. In the nucleus, the androgen receptor complex binds to a specific region of DNA known as the hormone response element, where it either up-regulates or down-regulates translation of specific genes. The effects of mediation by the androgen receptor complex are highly variable (Alonso & Rosenfeld, 2003).

It is widely believed that DHT is the major cause of baldness. Paradoxically, individuals with a genetic predisposition to baldness have an opposite response in some of their pilosebaceous units. In such individuals, the units in the scalp revert from being thick and darker in color, to being thin and lighter. However, the exact mechanism as to how DHT causes baldness remains to be determined. Furthermore, we have yet to understand why DHT acts in a paradoxical manner when affecting hair growth, creating baldness in some areas while stimulating new hair growth in other areas.

Although no definitive theories exist, Ustuner (2013) has proposed that gravity might play a role in causing baldnesss. He seeks to explain the unique pattern of manifestation in effected individuals by which is hair loss starts at the temples and the vertex of the scalp and proceeds to other areas. Ustuner believes that the weight of the facial tissue, which is supported by the scalp, causes damage to the hair follicles after puberty. During youth, there is sufficient fat tissue under the skin, which acts as a buffer to the hair follicles, and protects them from scalp pressure. However, this buffer gets diminished after puberty. The increase in DHT levels causes the fat layer to becomes increasingly thinner, essentially losing its buffering ability. As a result, pressure in the hair follicles increases causing follicular damage, eventually leading to hair loss. He notes that women have a lower incidence of balding, due to the effect estrogen has on maintaining the subcutaneous fat, thereby preventing baldness until at least menopause (when estrogen levels decrease) (Ustuner, 2013).

Ustuner continues to say that resulting increase in pressure weakens the follicle. To compensate for the increased pressure, the follicle sequesters more DHT more testosterone to make the hair follicle stronger. As a result, the subcutaneous fat layer becomes even thinner, creating a vicious cycle. Ultimately, the hair follicle becomes smaller and smaller, resulting in greater amounts of hair loss. According to this theory, areas in the front of the scalp should have greater pressure, due to greater amounts of soft tissues (the face) pulling down the scalp. This is consistent with the actual pattern observed (Ustuner, 2013).

One should be skeptical about this theory for several reasons. First, Ustuner does not explain the mechanism of how testosterone decreases the subcutaneous fat in the scalp. Even though there is a correlation between an increase in testosterone and its derivatives and a decrease in subcutaneous scalp fat following puberty, it is not proof of causation. Additionally, Ustuner does not address why or how testosterone causes an increase in hair growth in axillary areas (paradoxical effect of DHT).

Prostaglandin D2 and their suspected role in Hair Loss

Recently, there has been a new hypothesis as to what causes baldness. Researchers have found that individuals with androgenetic alopecia have elevated levels of prostaglandin D2 (PGD2) in areas of the scalp that are balding, while not having elevated PGD2 levels in haired areas of the scalp in the same individual. They note that in normal individuals, PGD2 is elevated in the catagen (regression) phase. This alludes to PGD2 having an inhibitory effect on hair, as the catagen phase is the transitional stage where the hair begins the process of falling out (Garza, et al., 2012). Additionally, when researchers targeted prostaglandin synthase (enzyme responsible for prostaglandin synthesis) in transgenic mice, the mice displayed symptoms characteristic of androgenetic alopecia due the increased synthesis of PGD2.

Genetic Factors

Many different genes have been suspected to play a role in hair loss. Thus far, most genetic studies investigating genetic causes have implicated the androgen receptor (AR) gene. This gene lies on the X chromosome, and its biological identifier is Xq11-12 (Ellis , et al., 2001). This is very intriguing, since as we have mentioned above, the most widely accepted cause of male pattern baldness is increased DHT levels. It would therefore be expected that the 5α–reductase gene be responsible for hair loss.

Treatments:

Finasteride (5α–reductase inhibitor)

In accordance with the theory that DHT is responsible for male pattern baldness, a 5α–reductase inhibitor should reduce male pattern baldness. Indeed, finasteride, a 5α–reductase inhibitor, has been one of only two FDA approved drugs used to treat hair loss. The recommended dosage for male pattern hair loss treatment is 1 mg/day taken orally. During trials conducted to determine the efficacy of finasteride in treating male pattern baldness, a placebo controlled study was conducted with 42 healthy participants. The trial demonstrated that administration of finasteride from 0.4 to 100 mg/day for up to 2 weeks significantly reduced the mean serum DHT from a baseline level. The reduction reached a maximum at the 1mg/day dosage. However, the study also found that 14 days after drug withdrawal, DHT returned to baseline levels. (Gormley, et al., 1990). In another study, individuals who were taking 1mg/day finasteride had a mean reduction in DHT of 68.4% versus the placebo (Kaufman , et al., 1998).

In the phase III placebo-controlled studies, the effects of finasteride on hair regrowth was assessed. Three studies were conducted in this phase; all were randomized, double-blinded and placebo controlled, and included 1879 male patients ages 18-41 years. All the individuals reported active shedding of hair at least 3 years prior to volunteering for the study. Hair loss among subjects ranged from mild to moderate. After categorically classifying subject's hair loss based on severity, they were given either a placebo or 1mg/day finasteride for 1 year. To detect whether or not finasteride increased hair growth, a baseline hair count was obtained before and after treatment, using macro-photographs of a 5.1cm2 area of the leading edge of the vertex with hair loss.

After analyzing these photographs, finasteride was found to cause significant progressive increase in hair counts in all areas of the scalp studied (vertex, mid and frontal) during 12 months of treatment. There was an 11% increase in hair count in subjects taking finasteride, compared to a 2.7% reduction in hair count during the same 12-month period in placebo subjects. Subjects taking finasteride for an additional year maintained their hair count, while those on the placebo continued to lose hair. After the 2-year period, 83% of those taking finasteride had no further hair loss, compared to only 28% of those taking the placebo. (Waldstreicher, et al., 1997).

Overall, tolerability was the same for both the placebo and finasteride receiving groups. The only difference was in sexual function disorders which were reported in a higher percentage in the group receiving finasteride. It should be noted that although there was a difference between both groups, the difference was relatively small, 3.8% in the placebo group and 2.1% in the group receiving finasteride. Additionally, of the subjects reporting sexual disorders during therapy, many cases were resolved even though they continued taking finasteride. All subjects who withdrew from the trial due to sexual disorders reported that the problems were resolved after discontinuing

the drug (Waldstreicher, et al., 1997). Based on these studies, it appears that finasteride does indeed reduce hair loss in most men. And since finasteride causes hair regrowth by altering DHT levels, it supports the DHT theory of baldness.

More research has to be carried out to determine whether there is a link between male breast cancer and the use of finasteride, as has been suggested by the UK Medicines and Healthcare Products Regulatory Agency. They have suggested that male breast cancer might be linked to the decrease in the ratio of testosterone to estrogen when taking finasteride. For individuals using finasteride to treat hair loss, this suggestion should be taken with a bit of skepticism, as a majority of cases of male breast cancer was found in males taking the 5mg/day dose as a form of treatment for benign prostatic hyperplasia. In contrast, only a small percentage of prostate cancer was reported in individuals taking the 1mg/day dose suggested for hair loss treatment (Shenoy & Prabhakar, 2010). However, as one must continue using the drug to prevent further loss of hair, there is a need for long term studies of possible side effects.

It should also be noted that non-Caucasian participants appeared to have less regrowth of hair compared to Caucasians. However, one has to be cautious in interpreting this, since only a small portion of the study subjects were non-Caucasian. To definitively state that non-Caucasians experienced less hair regrowth, future studies conducted must include a representative number of non-Caucasian subjects.

Dutasteride

Similar to finasteride, dutasteride is also a 5α–reductase inhibitor, however, dutasteride inhibits not only type II, but also type I forms of 5α–reductase. Scientists don't exactly know the effect type I 5α–reductase inhibitor has on hair loss, since no deficiency has been found for it. However, evidence suggests that dutasteride is three times more potent than finasteride in inhibiting type II, and more than 100 times more potent in type I enzyme. (Clark , et al., 2004). This would suggest that dutasteride would have a greater ability preventing hair loss, and promoting hair regrowth. Indeed, dutasteride has been found to decrease serum levels of DHT by more than 90% when compared to only 70% in finasteride. (Dallob, et al., 1994).

In 2002, the FDA approved dutasteride as a treatment for benign prostatic hyperplasia. Phase trials were also conducted on its use as a treatment for male pattern baldness. During the phase II trails, researchers found increased hair growth on the scalp. They also found that a 2.5mg dosage of dutasteride was more effective than a 5mg dosage of finasteride (Olsen , et al., 2006). However, the phase III trials were put on hold due to concerns of side effects. For this reason, one should be hesitant in using this drug.

Minoxidil (Vasodilator)

Minoxidil, the second of only two drugs the FDA approved for treating hair loss, was initially developed as a treatment for ulcers. When minoxidil was applied to dogs during the trail phase, the ulcers did not improve, however, minoxidil was found to be a strong vasodilator. As a result, the FDA approved minoxidil tablets as a treatment for high blood pressure in 1979 (Conrad , 2008). Initially, when studies on the effectiveness of minoxidil as a vasodilator in humans were conducted, researchers found unexpected hair growth. That is when it occurred to researchers that minoxidil might be an effective treatment for hair loss (Gilmore, et al., 1970)

Although the mechanism of minoxidil's ability to cause hair growth is poorly understood, some theories have been suggested. Minoxidil might increase hair growth by either shortening telogen or prolonging the anagen phase. Others propose that minoxidil is a potassium channel opener. When the potassium channel opens, it causes a hyperpolarization in cell membranes, this widens the blood vessels surrounding the hair follicle, thereby allowing more oxygen and nutrients into the follicle (Goren, et al., 2015).

In the 1980s, studies reported that a 2% topical solution of minoxidil reduced baldness in about 50% of patients. Although the study found that few mature terminal hairs were regrown, the number of fine, non-pigmented villus hairs were reduced. These studies acknowledged that the ideal candidates for minoxidil therapy were those who had been bald for less than five years, and whose baldness was less than 10cm in diameter and located on the vertex. They found that minoxidil was not useful for frontal hair loss. Additionally, patients who discontinued treatment showed a rapid loss of the newly regrown hair. After 3 months of discontinuing therapy, their hair count was below baseline levels (Savin , 1987).

After getting approved by the FDA as a treatment for hair loss in men, minoxidil became available under the name Rogaine, and was obtainable only by prescription. In 1996, Rogaine was approved for over-the-counter sale, and for the production of generic versions. Eventually a 5% topical solution was approved by the FDA (Conrad , 2008).

Two studies have been conducted comparing the 2% versus the 5% solution. One of the studies measured hair mass before and after a two-year period. This study found a greater hair mass in individuals taking the 5% solution. The difference was most apparent early in the study. After taking the 5% solution for five months, these individuals experienced a 55% increase in total hair mass compared to the baseline, while those on the 2% solution only experienced a 25% increase. After the end of the 2-year

period, the 5% group had an increase of 25% over the baseline, while those on the 2% had only 15% (Price, 1996). The second study found that the 5% minoxidil produced a significantly greater amount of non-villus hair by count as compared to the 2% group. They also found that patients using the higher dosage had an increased likelihood of noticing more hair coverage on the scalp in their assessment of treatment benefits (Trancik, 1998).

Generally, minoxidil is well tolerated, however, some side effects have been reported. The most frequent side effect is itching, redness, or irritation of the scalp in the treated area. Unwanted hair growth elsewhere on the body has also been reported. Some individuals reported exacerbation of hair loss after applying minoxidil.

Severe allergic reactions have also been reported including, rash, hives, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue, chest pain, dizziness, fainting, tachycardia, headache, sudden and unexplained weight gain, and swelling of the hands and feet (Rogain Side Effects, 2015). Overall, it seems safe to say that minoxidil is a somewhat effective treatment for male pattern hair loss, however, compared to finasteride, it is less effective but has the advantage of being a topical.

Ketoconazole (anti-fungal)

Ketoconazole, a common anti-fungal shampoo widely used in treating seborrheic dermatitis (dandruff), has also been suggested to contain hair regrowth properties. Recently, a study demonstrated that individuals applying a 2% solution of ketoconazole produced comparable levels of hair growth when compared to those using 2% minoxidil. Both groups achieved greater levels of hair growth compared to those using un-medicated shampoo (Pierard-Franchimont, et al., 1998). Similar results were obtained when treating model mice with androgenetic alopecia with 2% ketoconazole, when compared to the placebo group (Jiang, et al., 2005).

Why ketoconazole causes hair regrowth is not clearly understood. Some have suggested that ketoconazole plays a role in local disruption of the DHT pathway. They have suggested that when used in combination with finasteride, it may have a greater affect in reducing DHT levels compared to using finasteride on its own. (Perez, 2004). Its effect on DHT has been used to explain why some individuals using ketoconazole experience gynecomastia (enlargement of the breasts) (Wolverton , 2002).

Clearly, it seems like ketoconazole has some hair regrowth abilities, however, there is a need for more studies to be carried out to demonstrate how effective it is compared to placebo and other treatments. Additionally, more research needs to be done on the mechanism behind how it causes hair growth. Finally, studies have to demonstrate whether or not using ketoconazole for an extended period of time is safe.

Low-level Light Therapy

Use of a ruby red laser (694nm) , resulted in increased hair growth in mice who had their backs shaved (Mester, et al., 1968). Although light therapy has been shown to effectively reduce inflammation, improve wound healing and reduce stroke symptoms, the mechanism behind it is poorly understood. Some have hypothesized that the light increases levels of ATP synthesis in the mitochondria. There has been evidence that there is an increase in activity in complexes II and V in the electron transport chain (Oron , et al., 2007).

Many light therapies are marketed as treatments for hair loss. Typically, these devices are brushes or combs that have a red light shining out of the tips onto the scalp. Such devices are available for purchase over the counter. So far only one such device has been approved by the FDA as not being harmful, but does attest to its ability to treat hair loss. Such approval is sought after, as using certain wavelengths of light can be harmful.

Companies have capitalized on the little research showing hair regrowth abilities and have produced light therapy devices for consumers, despite the little research that has been conducted to determine the safety and efficacy of it. As a consumer, one must therefore be very skeptical of buying such light therapy devices. More research must be done before anything definitive can be said about using light therapy to treat hair loss.

Discussion

In light of research indicating that drugs can prevent further progression of hair loss, we may effectively conclude that hair loss can be treated. We cannot however conclude that hair loss can be cured, for a cure implies a reversal of a problematic medical phenomenon. Hair loss as of yet cannot be cured because the effectiveness of hair loss drugs is only for as long as they are administered. To find a cure, a definitive theory explaining the causative mechanisms behind hair loss is necessary. Once we understand the mechanism behind hair loss, most certainly a cure will follow.

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Vitamin D Deficiency and Suicide

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Abstract
Vitamin D deficiency, in an increasingly modernized world, is a major global health issue and so is major depressive disorder (MDD) and its high fatality risk. Studies suggest that there may be a connection between the two. Several studies have found a connection between low levels of vitamin D and higher rates of major depressive disorder, depressive symptoms and suicidal ideation. Specifically, lower concentrations of vitamin D was seen in the subgroup of patients with suicidal thoughts when comparing with non-suicidal depressed patients. A likely reason for this may be the well- researched role vitamin D plays in regulation of inflammatory cytokine markers in the brain. Elevation of these proinflammatory cytokines is shown to be a major contributing factor to depression and suicidality. Therefore, a lack of vitamin D contributes to an increase in inflammation and thereby an increase in the risk of depression and suicide. Thus, increasing vitamin D levels by supplementation or sun exposure, may decrease depressive symptoms. Because this research is recent, there are few studies assessing the possible benefits and limitations of using vitamin D as a treatment method.

Introduction
Vitamin D_3 is a fat soluble vitamin, stored mainly in the liver and adipose tissue. It is primarily obtained by the conversion of cutaneous 7-dehydrocholesterol to previtamin D_3 by sunlight, specifically Ultraviolet (UV) B radiation with a wavelength of 290–320 nanometers. Previtamin D_3 then spontaneously isomerizes to vitamin D_3, or cholecalciferol. Vitamin D3 is biologically inert and must undergo two hydroxylation to be biologically active. The first hydroxylation occurs in the liver, by 25-hydroxylase, converting vitamin D3 to calcidiol, or 25-hydroxyvitamin D [25(OH)D]. The second hydroxylation occurs by 1α-hydroxylase, found primarily in the kidneys, although recent research found this enzyme in many other tissues. The second hydroxylation converts the 25-hydroxyvitamin D to the biologically-useful calcitriol, or 1,25-dihydroxyvitamin D [1,25(OH)2D] (Vitamin D: Fact, 2016).

Besides for obtaining vitamin D by sun exposure, vitamin D can be obtained by food. However, very few foods in nature contain vitamin D. Therefore, a better way of obtaining vitamin D is from cholecalciferol-fortified foods or supplements (Vitamin D: Fact, 2016).

Serum concentration of 25-hydroxyvitamin D is the best indicator of vitamin D status, because it reflects vitamin D obtained by sun exposure and ingestion. It also has a fairly long circulating half-life of 15 days and its concentration is not affected by other metabolites. However, it does not indicate how much vitamin D is stored in body tissues. In contrast, 1,25-dihydroxyvitamin D has a short circulating half-life of 15 hours and its levels are closely regulated its own concentration and by hormones and electrolytes like parathyroid hormone, calcium, and phosphate. Additionally, levels of 1,25(OH)2D only decreases when vitamin D deficiency is severe. Therefore, 25-hydroxyvitamin D levels are used to assess sufficiency (Vitamin D: Fact, 2016).

There is considerable discussion of what serum concentrations of 25-hydroxyvitamin D indicate vitamin D deficiency, adequacy for bone health and optimal health overall. As of now, there is no specific cut points established by a scientific consensus process. However, the committee of the Institute of Medicine, based on its review of data of vitamin D needs, have concluded that there is a risk of vitamin D deficiency at serum 25(OH) D levels of less than 30 nmol/L (<12 ng/mL). Additionally, some people are potentially at risk for inadequacy at concentrations ranging from 30-50 nmol/L (12–20 ng/mL). They stated that 50 nmol/L is the serum 25-hydroxyvitamin D level that is adequate for 97.5% of the population. Only at concentrations of greater than 125 nmol/L (>50 ng/mL) is there potential adverse effects of overdose (Vitamin D: Fact, 2016).

Cutaneous vitamin D levels are mainly dependent on climate, overall skin exposure, skin melanin content and age. Residing in a climate closer to the equator results in more year-round sun exposure and more vitamin D synthesis. Although surprisingly, geographic location does not consistently predict average serum levels in a population. Opportunities exist to form vitamin D from exposure to sunlight during the spring, summer, and fall months even in the far north or south, and it can be stored in fat for periods of little sunlight. Darker skin absorbs less sunlight and, therefore, more melanin reduces the production of vitamin D. Perhaps, this characteristic may contribute to the consistency in average serum levels of those near the equator and those further away, because those who live near the equator generally have a darker skin tone that synthesis less vitamin D than those farther away with a lighter skin tone. Vitamin D synthesis may also decline with age because skin synthesis declines (Johnson, 2016).

Major depressive disorder (MDD) is a common disorder and leading source of disability worldwide. It is a major global health problem, with more than 50% of all suicides contributed to clinical depression (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011). Classically, the prevailing hypothesis on the cause of major depression was a deficiency of monoamines, a class of neurotransmitters and neuromodulators, like serotonin. Therefore, current available medications for major depression mainly target monoamine pathways. However, research has not shown a consistent relationship between serotonin and depression

(Gardner & Boles, 2011). And although relatively affective, 30% of depressed patients do not achieve remission even with multiple, monoamine-regulating, treatment trials (Miller, Maletic, & Raison, 2009). Additionally, 50% to 80% of all patients treated for major depression will experience relapse (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011). These monoamine regulating drugs can have serious side effects, which include worsening of depressive symptoms and suicidal ideation. Therefore, it is imperative to find novel pathways involved in depression and consequently more effective treatments for those suffering.

Historically, in literature, and in art and religion, happiness and positivity have been associated with summertime, daylight, and sunny and open landscapes. In contrast, fear and gloom have been associated with winter, night and dark and polluted urbanscapes. At the height of early industrialism, in such polluted cities, rickets, which is extreme vitamin D deficiency was first recognized. Among the symptoms of rickets, mental symptoms were also described and Florence Nightingale stated: "People say the effect [of sunlight] is on the mind. So it is, but the enlightened physician tells us it is on the body too." (Humble, 2010) Mental disturbances caused by reduced sun exposure seemed self-evident that no one seemed to note the mental health benefits of rickets treatment, which was primarily to increase sun exposure. Because of the awareness of adequate sun exposure, rickets disappeared as a public health problem. Nevertheless, from the 1950s and on, with increasing modernization, the time spent indoors increased for all ages worldwide. Additionally, since the 1980s, the public is being warned about the dangers of UV rays and its contribution to malignant melanoma. Therefore, people have developed concern of excessive sun exposure and are applying sunscreen and covering up more when they are outside. At the same time, as these changes are taking place, the incidence of depression, especially in children and adolescents, has become more and more prevalent in the United State and Europe (Humble, 2010). Additionally, it is a long, well-known and perplexing observation that death by suicide is highest in the springtime for people living in temperate climates, which is also when vitamin D levels are the lowest in the population (Umhau et al., 2013). Is there a correlation between these two observed phenomena of depression and vitamin D synthesis, and what would be there relationship?

Materials and Methods

Materials for this comprehensive review were obtained from Touro College's online library, PubMed, PubMed central, Proquest, National Institute of Health website and Merck Manual website. The material consisted of clinical research papers, peer-reviewed journal articles and clinician-directed informational reports. The material was reviewed, critically analyzed and compiled to answer research questions.

Discussion

Vitamin D and Inflammation:

Classically, Vitamin D is associated with bone health. Vitamin D aids calcium absorption in the gut and maintains adequate serum calcium and phosphate levels for normal mineralization of bone (Vitamin D: Fact, 2016). It is also needed by osteoblasts and osteoclasts bone growth and repair. Vitamin D deficiency was mostly associated with rickets and osteomalacia at extreme deficiency. Rickets is a disease in children where vitamin D deficiency leads to bones not being able to properly mineralize, which results in soft bones and skeletal deformities. In adults, vitamin D deficiency can lead to osteomalacia, or weak bones (Vitamin D: Fact, 2016).

Recently, many other functions of vitamin D have been discovered and vitamin D receptors have been found in many tissues. One of the most significant findings is the function of vitamin D in immune system modulation. There is an increasing number of studies that demonstrate the importance of vitamin D in the reduction of inflammation and the association of vitamin D deficiency with increased inflammation (Peterson & Heffernan, 2008). Most of the known effects of vitamin D are facilitated through vitamin D receptors (VDR). These vitamin D receptors have been found extensively in immune system cells, specifically in T lymphocytes and macrophages. Furthermore, macrophages have been found to express 1α-hydroxylase, which is the enzyme that is responsible for the final step in synthesizing biologically-active vitamin D. Additionally, they have been found to express 24-hydroxylase, the major degrading enzyme of vitamin D. This means that these cells can regulate the production and secretion of vitamin D in their own vicinity (Peterson & Heffernan, 2008).

Additional evidence now suggests that vitamin D insufficiency may play a role in immune system dysfunction and low 25-hydroxyvitamin D serum levels are shown to be associated with autoimmune diseases like multiple sclerosis, Type 1 diabetes and rheumatoid arthritis. Recently, this insufficiency has been correlated to macrophage dysfunction, such as impaired chemotaxis, phagocytosis and increased production of proinflammatory cytokines. Proinflammatory cytokines are produced predominantly by activated macrophages. These cytokines take part in cell signaling and up-regulation of inflammatory reactions. Vitamin D has been shown to reduce inflammation by down-regulating the expression of monocyte toll-like receptors (TLRs), which are known to activate inflammation and aggravate autoimmune disease and sepsis (Peterson & Heffernan, 2008).

In light of the research, Peterson and Heffernan (2008) sought to investigate the relationship between serum vitamin D status (25(OH)D) and inflammatory markers in 69 healthy women.

They recruited people with high UVB and minimal UVB exposure, as assessed by a screening questionnaire, to obtain a range of serum levels. Volunteers were excluded for multiple factors, including, if they took a vitamin D supplement, had a current or previous medical condition or took medication affected immune function. The results revealed that serum TNF-α, a proinflammatory cytokine, was significantly lower in the group of women with high UVB exposure, after controlling for multiple factors. However, there was no significant statistical difference shown between the two groups in regards to other proinflammatory cytokines, like IL-10, CRP, IL-6. Serum TNF-α concentrations are increased in several diseases like multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, heart disease and osteoporosis, and lowering TNF-α concentrations through sufficient vitamin D levels may improve disease outcomes. The main limitation of this study was the small sample size. Additionally, women who frequently tan are characteristically different from non-tanners and may engage in behaviors that can affect the study outcomes. This study shows a negative correlation between vitamin D status and TNF-α concentration in healthy individuals. More research is needed to determine if vitamin D can be used as a therapy in inflammatory diseases.

A case where vitamin D deficiency seems to contribute to immune modulation, is in diabetic foot infection. Diabetic foot infection is a reflection of a diabetic patient's altered immune system. In an infectious state, pro-inflammatory cytokines are released in the body, such as IL-1β, IL-6, interferon-γ (IFN-γ) and TNF-α, or chemokines such as IL-8. To counteract these factors and avoid a hyper-inflammatory state, the body releases anti-inflammatory cytokines such as IL-10. In a diabetic patient, several factors lead to decreased wound-healing abilities, including impaired cytokine production. This abnormal wound healing is attributed to immune dysregulation because of vitamin D deficiency in addition to hyperglycemia (Timms et al., 2002).

Another study, investigating the relationship between vitamin D deficiency and increased inflammatory cytokines, was done by Timms et al. (2002), by evaluating the influence of vitamin D on the concentrations of IL-1β, TNF-α, IFN-γ and IL-6 in patients with diabetic foot infection. Subjects were 112 diabetic patients with diabetic foot infection while 107 diabetic patients with no evidence of infection served as controls, additionally, 40 healthy subjects were included in the study. Vitamin D deficiency, a serum 25-hydroxyvitamin D < 50 nmol/l, was found in 71.4% of the diabetic foot infection patients, 61.6% of the diabetic controls and 48.6% of the healthy volunteers, but severe deficiency, with a serum 25-hydroxyvitamin D of < 25 nmol/L, was mostly found in diabetic foot infection patients than in diabetic controls and healthy volunteers. In both patients and controls, 25-hydroxyvitamin D levels was found to be significantly negatively correlated with IL-1β as well as IL-6 levels and moderately negatively correlated with TNF-α levels, but not correlated with IFN-γ levels. In this study, diabetic patients with foot infection serve as a model for immune system abnormality resulting from hyperglycemia and infection, with increased pro-inflammatory cytokine concentrations. The results of the present study showed that elevated cytokine responses occur as a factor of vitamin D deficiency in patients with diabetic foot infection. Vitamin D deficiency, particularly when serum levels were very low, intensified inflammatory cytokine release in patients with diabetic foot infection.

Because of this increasingly strong correlation and possibly causation of vitamin D deficiency to amplified inflammation, and to try to prove causation more visibly, Hoe et al. (2016) recently performed a study ex vivo. They studied the effects of 1,25-dihydroxyvitamin D, the active metabolite of vitamin D, on peripheral blood mononuclear cells (PBMCs) and purified immune cell subsets isolated from healthy patients, when they exposed those cells to stimulation with Gram-positive heat-killed pneumococcal serotype 19F (HK19F) and Gram-negative Escherichia Coli serotype 055:B5 derived lipopolysaccharide (LPS) bacterial ligands. They found that in 1,25(OH)2D3 pretreated samples compared to non-treated, for both ligands, 1,25(OH)2D3 suppressed proinflammatory cytokines, like TNF-α, IFN-γ and IL-1β, and chemokine IL-8, in peripheral blood mononuclear cells (PBMCs) and TNF-α and IL-1β in CD14+ Monocytes. Anti-inflammatory IL-10 was increased in HK19F-stimulated monocytes. Additionally, when comparing the blood samples of the healthy subjects, they found that levels of HK19F-specific IFN-γ were significantly higher in vitamin D-insufficient adults (<50 nmol/L) compared to sufficient adults (>50 nmol/L). This study suggests Vitamin D causes modulation of inflammation of the immune system response which is critical for host defense.

Inflammation and Depression

The progression and complications of many diseases and disorders, like cardiovascular disease, diabetes and cancer, are being increasingly attributed to inflammatory dysfunction. Additionally, this theory of disorder is being extended to neuropsychiatric disorders, with mounting evidence suggesting that defective alterations of the immune system seen in psychiatric patients may contribute to their disorders, and with mounting evidence of inflammation contributing to major depressive disorder. Patients with major depression have been found to exhibit elevated proinflammatory cytokines, like IL-1β, TNF-α and IL-6, which have been shown to interact with areas known to be involved in depression, including neurotransmitter metabolism, neuroendocrine function, and neural plasticity. Furthermore, psychosocial stress, a common precipitator to major depression, has been shown to have the ability to stimulate inflammation, owing to

the fact that it activates sympathetic nervous system pathways. Moreover, depressed patients with increased inflammatory markers have been found to have greater resistance to treatments, and in studies, antidepressant drugs have been found to be correlated with decreased inflammation (Miller, Maletic, & Raison, 2009).

Recurrent coinciding comorbidities and drug efficacies suggest that depression is part of a group of related conditions, all of which are associated with inflammation dysfunction. They are sometimes referred to as the "affective spectrum disorders", and include migraine, irritable bowel syndrome, chronic fatigue syndrome, fibromyalgia and generalized anxiety disorder, among many others. Based on current knowledge, these diseases, including depression, seem to be a function of three connected abnormalities: monoamine dysfunction, increased inflammation, and mitochondrial disorder (Gardner & Boles, 2011)

To test if inflammation may correlate or even cause major depression, several studies performed on animals who have been injected with the endotoxin lipopolysaccharide (LPS) to generate inflammation, showed that these subjects subsequently developed depressive-like symptoms. In clinical practice, it has been seen that patients treated with interferons and interleukins, to promote inflammation against certain forms of cancer and viral infections, like Hepatitis C, frequently develop depression. To further investigate this relationship Bay-Richter, Janelidze, Hallberg, & Brundin (2011) induced inflammation in rats by administering Escherichia coli serotype 055:B5 lipopolysaccharides (LPS) intraperitoneally for four days. They measured their levels of cytokine markers at the state of sickness and then at the state of depressive-like behaviors. They also measured mRNA transcription of cytokines in specific brain areas related to depression as well as levels of cytokines in serum and cerebral spinal fluid. Cyclooxygenase enzymes, inflammatory stimulating COX-1 and COX-2, have previously been shown to correlate with depressive-like behavior, thus, it is suggested that proinflammatory cytokines can induce depressive-like behavior by activating prostaglandins, and inflammation, through the cyclooxygenase enzyme. Therefore, they also measured cyclooxygenase enzymes in the brain of the mice. This is the first published study examining the relationship between proinflammatory cytokines and cyclooxygenase enzymes in the blood, CSF and depression-related brain areas and their capacity to cause sickness and behavioral changes.

In this study, they found that there was a clear distinction between the phase of sickness, which is 2 hours after injection of lipopolysaccharides, and the phase of depressive symptoms, which is 24 hours after injection, for cytokines, cyclooxygenase enzymes and mRNA transcription of cytokines. During the sickness behavior, IL-1β and IL-6 were elevated in the blood, IL-1β was elevated in the CSF, and TNF-α was elevated in the striatum of the brain. Also, IL-1β mRNA transcript was increased in the frontal cortex and hippocampus. Curiously, in this phase, IL-6 and COX enzymes were decreased in the hippocampus. After 24 hours of injection, the rats did not show signs of sickness but began displaying depressive symptoms in the forced swim test. This test is used on rodents to evaluate depression and is used when assessing antidepressant drugs and therapies. During the depressive phase of behaviors, the transcription of TNF-α mRNA, IL-6 levels and COX enzymes levels shifted back to normal levels in all brain areas, but the cytokine IL-1β was still significantly elevated in the frontal cortex and hippocampus and there was an increase in the concentration of IL-1β in the cerebral spinal fluid. This increase was only found in the CSF and not in blood serum. This may explain why studies measuring the serum level of cytokines in patients with major depressive disorder, did not find any significant difference when comparing depression levels with normal levels. In this study, they found that Lipopolysaccharide-induced depression is primarily associated with the increase of IL-1β. This cytokine remains elevated in the brain even after the initial inflammatory phase and continues to contribute to a depressive-like phenotype in the rats. This suggests that a short term immune inflammatory response may lead to long term changes in transcription of inflammatory markers and that transcription levels may even increase after the initial immune response (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011).

Although, elevated inflammatory markers are found in many patients with psychiatric disorders, there is no clear differentiation or explanation why some patients have elevated cytokine concentrations and some do not. Perhaps, there is a specific subgroup of patients that will display signs of inflammation. To advance and investigate if the phenomenon of increase cytokine concentrations in psychiatric patients is specific to a subgroup of depressed patients, Janelidze, Mattei, Westrin, Traskman-Bendz, & Brundin (2011) designed a study dividing their subjects by non-suicidal and suicidal patients. In this study, they compared plasma cytokine levels in 47 treated and untreated patients with depression that had attempted suicide, 17 untreated patients with a diagnosis of major depressive disorder that did not have any suicidal ideation and 16 somatically healthy controls with no current or previous history of neuropsychiatric disorder. They found statistically significant elevated plasma concentration of proinflammatory cytokines IL-6 and TNF-α and decreased plasma concentrations of anti-inflammatory cytokine IL-2 in the suicide attempters, compared to the non-suicidal depressed patients and healthy controls. Elevated cytokines were seen in suicidal patients, regardless if they had received treatment or not. Although a limitation of this study is that the duration of the disorder and duration of treatment were not considered. Interestingly, these patients with elevated cytokines did not show any signs of systemic or local

infection or inflammation. This suggests that inflammation seen in depressed patients is specific to those that are suicidal, although it may also be specific to different subgroups of depression too. Furthermore, it suggests that these elevations are associated and may contribute to suicidal ideation. Because this study used patients with untreated major depression, one group it did not control for specifically, is patients with treatment-resistant depression, and therefore, perhaps increased cytokines is seen in all patients with treatment-resistant depression, not only those who have attempted suicide. Evidence of abnormal cytokine elevations were observed in suicidal patients, compared to non-suicidal depressed patients and healthy controls, and may contribute to suicidality.

As the mechanism that causes depression and suicide is located in the brain, it is important to understand if these systemic inflammatory changes relate to changes in the central nervous system. Lindqvist et al. (2009) tested the proinflammatory cytokine profile of the cerebral spinal fluid (CSF) of 63 institutionalized patients who had recently attempted suicide compared to healthy controls. They also evaluated if there was a difference in the cytokine profile of patients who had attempt a violent method of suicide compared to the patients who used a nonviolent method. The patients underwent a "wash-out" period where they discontinued all antidepressant and antipsychotic medication until there was no trace of it in their blood. Depressive symptoms were assessed using the Montgomery-Åsberg Depression Rating Scale (MADRS) and suicidal ideation was assessed using the Suicide Assessment Scale (SUAS). They found that the patients who had attempted suicide had higher levels of cytokine IL-6 in their cerebral spinal fluid to healthy patients, and the violent suicide attempters had the highest concentration of IL-6. They also found that CSF IL-6 had a positive correlation with intensity of depressive symptoms in all suicidal patients. Additionally, they found that IL-6 levels correlated positively with current suicidal ideation and suicidal symptoms, as in patients expressing a "wish to die" and "preoccupation with suicidal thoughts." These results suggest that, as discussed previously, IL-6 may have specific relevance to suicide. They did not find any significant difference in proinflammatory cytokine IL-1β, TNF-α, and IL-8 between the groups studied. Lindqvist et al. then went further to try to determine a mechanism of how these abnormalities may contribute to suicidality. They compared levels of cytokines with known biomarkers of depression and suicide and found that both IL-6 and TNF-α was related to increased levels of 5-HIAA, which is a metabolite of serotonin, and HVA, which is a metabolite of dopamine, in the fluid of the patients who attempted violent suicide methods. This suggests that proinflammatory cytokines may modulate monoamines in the central nervous system. Further, longitudinal studies would be helpful in studying the effects of cytokine and monoamine fluctuation and its neuropsychiatric effects. In summation, Lindqvist et al. found that inflammatory cytokines were directly correlated with the presence and extent of suicidal thought and behavior and the degree of violence of a suicide attempt.

Vitamin D and Depression

As discussed previously, there is strong evidence that vitamin D deficiency may be contribute and cause immune system dysfunction, including elevated inflammatory markers. And elevated inflammatory markers may play a role in depression with suicidal ideation. Therefore, there are many that suggest that vitamin D deficiency may be a contributing factor in those who suffer from suicidal thoughts accompanying major depressive disorder.

The presence of vitamin D receptors (VDR), in the central nervous system was first discovered in 1982 by Stumpf, Sar, Clark, and DeLuca. And nowadays, there is significant evidence of vitamin D's presence and actions on different parts of the brain and nervous system functioning. There is increasing evidence that the vitamin D receptor (VDR) mediates transcription of more than 1,000 genes (Umhau et al., 2013). Calcitriol is known to function in the regulation of neurotransmitters, specifically dopamine, adrenaline, noradrenaline and acetylcholine, regulates several neurotrophic factors of the central nervous system, by enhancing nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF), and assist with anti-oxidative properties in the brain (Humble, 2010). Many of the effects that vitamin D has on the brain, share pathways with factors, when abnormal, are known to be associated with several neuropsychiatric disorders including major depressive disorder (Humble, 2010). Based on this, inadequate levels of vitamin D may lead to changes in the central nervous system that may interfere with brain function and contribute to mood disorders.

As depression is underdiagnosed in non-institutionalized healthy populations and subclinical depression and chronic low mood is common in older adults, and is a factor in morbidity, mortality and quality of life, one study seeking to clarify if vitamin D may be associated with depression in healthy adults was done by Lee et al. (2011). Lee et al. used baseline data from the European Male Ageing Study (EMAS), a cohort study of male aging in Europe, to determine whether concentrations of serum 25-hydroxyvitamin D were associated with levels of depression in a large sample size of 3151 subjects, well-characterized, community-dwelling sample of middle-aged and older men with a mean age 60 ± 11. Subjects were assessed on social issues, lifestyle behaviors, and any related comorbidities, like heart conditions, pituitary disease, diabetes, and cancer. Depressive symptoms were assessed using the Beck Depression Inventory-II (BDI-II). Their results found that serum vitamin D concentrations were significantly lower in men with depressive symptoms, scoring ≥14 on the

BDI-II, 10 nmol/L decrease in 25-hydroxyvitamin D was associated with an average increase of 5.2% in the BDI-II score. Even after adjusting for age, smoking, alcohol consumption, physical activity, BMI, comorbidities and adverse life events, there was still a significant difference in serum vitamin levels. Additional adjustment for season did not change this inverse association. Only 22 men (0.7%) reported taking vitamin D supplements and exclusion of these men did not change the results. A major limitation of this study, is its difficulty in assessing causality or even if vitamin D contributes to depression. It may be that vitamin D insufficiency may just be a 'risk marker' of general poor health and lifestyle. Depressive behaviors of decreased physical activity and exposure to sunlight may by itself lead to lower vitamin D levels. Lee et al. did attempt to adjust for this factor by assessing if physical activity can contribute to low vitamin D levels but this adjustment does not measure sunlight exposure or rule out reverse causality. The main strengths of this study is their large sample size of healthy subjects of similar origin and uniform methods of assessing depressive symptoms. Their study did find an inverse relationship between serum vitamin D levels and depressive symptoms, mostly independent of confounding factors.

To further this relationship and see if low vitamin D levels is a conclusively a predisposing factor of suicide, Umhau et al. (2013), did a case-control study of 25-hydroxyvitamin D concentration, of serum samples stored in the Department of Defense Serum Repository, of deployed active-duty service members who had subsequently committed suicide. 495 subjects were selected from the Armed Forces Health Surveillance Center (AFHSC) who had officially verified suicides occurring between 2002 and 2008 and had blood sampled within 24 months of death. Controls were randomly selected from the same data bank, and were matched as taking the blood work within 12 months of those who had committed suicide, to minimize for any temporal changes in military environment. They found an increased risk of suicide in lower levels of serum 25-hydroxyvitamin D, and all those who committed suicide has a seasonally adjusted levels below 20 ng/mL. Additionally, they found that 30% of the suicides assessed occurred in those in the lowest levels of vitamin D status. When they graphed the data, they found a curve that is characteristic of nutritional intake, which appears to have benefit until a threshold is reached and after that, any additional enrichment shows little benefit. Meaning, that if sufficient nutrient input is reached then further input will have no effect on outcomes. Which is important when considering Vitamin D supplementation as a treatment method, as discussed later. One limitation of this study, is that vitamin D levels were not measured at the time of suicide but sometimes months before. This problem is lessened by the fact that vitamin D levels are shown to be correlated up to 3 years apart. Perhaps, active-duty service members may have a higher risk of suicide because they often work at night and are excessively covered up in the daytime, therefore, their reduced sun exposure and subsequent reduced vitamin D synthesis contributes to their suicidality. In this study, vitamin D status before suicide attempt was inversely correlated with suicide risk in a large sample size of a specific subset of the population with a general high risk of suicide.

Suicidal ideation may be sudden and in most cases, the first thoughts of suicide often occur less than 10 minutes before the suicide attempt. In a young, and somewhat aggressive, population like military service members, impulsivity may contribute to their risk of suicide. There seems to be a link between vitamin D and impulsivity, as vitamin D increases proinflammatory cytokines in the brain. Proinflammatory cytokines reduces serotonin activity which is long associated with impulsive suicide (Umhau et al.,2013). As discussed in detail earlier, perhaps, this effect of vitamin D is the mechanism by which vitamin D deficiency contributes to higher suicide rates.

Is present vitamin D concentration associated with suicide attempt? As a similarly designed study discussed previously, done by Janelidze, Mattei, Westrin, Traskman-Bendz, & Brundin (2011) to show that increased inflammatory cytokine concentration of IL-6 and TNF-α contributes to suicide risk, Grudet, Malm, Westrin, & Brundin (2014), takes it a step further, to see if vitamin D concentrations, which also contributes to increased inflammatory cytokine concentrations, differ in suicidal depressed patients compared to non-suicidal depressed patients. They included a total of 59 patients who had attempted suicide, and had two control groups of 17 untreated patients with major depressive disorder who had no suicidal ideation and 14 somatically and psychiatrically healthy patients. They found a significant difference in serum 25-hydroxyvitamin D levels between groups, with the suicide attempters have a significantly lower mean vitamin D concentration, of 47 ± 20 nmol/L, than the non-suicidal patients, 62 ± 27 nmol/L, and healthy patients, 65 ± 26 nmol/L. Additionally, 58% of patients in the suicidal group had a clinical vitamin D deficiency of below 50 nmol/L, compared to 30% in the other two control groups. When comparing vitamin D levels and inflammatory cytokines in all groups, they found a correlation between 25-hydroxyvitamin D serum concentrations and proinflammatory cytokine serum concentration of IL-1β. They did not find a statistically significant correlation between vitamin D and cytokines IL-6 and TNF-α in all groups. When comparing individual groups, they found an inverse association between 25-hydroxyvitamin D and IL-1β in the patients who attempted suicide and an inverse association between 25-hydroxyvitamin D and IL-6 in the non-suicidal depressed patients. In conclusion, they found a negative correlation between vitamin D levels and suicide risk and a negative correlation between vitamin D levels and proinflammatory cytokines. The cross-sectional design of

this study does not allow any conclusions whether low vitamin D status can be a direct contributing factor of suicide, but based on previous knowledge of the mechanism of 1,25-dihyroxyvitamin D on brain function and regulation, there is considerable evidence to suggest a direct causality.

Vitamin D deficiency is common in the modern world and Vitamin D supplementation is relatively inexpensive nutritional additive. If this vitamin D insufficiency has a direct contribution on depression and suicide, can vitamin D be used a treatment to reduce depressive symptoms and stop the epidemic of depression and suicide worldwide?

As a vitamin D intake is an easy nutritional therapy, Bertone-Johnson et al. (2011) seeks to clarify if vitamin D supplementation can prevent or treat depression. They patients used for this study were part of a large and diverse sample size that was tracked for a long period of time. The subjects used for this study were part of the observation study (OS) component of the Women's Health Initiative (WHI) This is a database composed by the National Institute of Health which allowed Bertone-Johnson et al. to follow the health of 81,189 postmenopausal women over an 8-year period. Baseline intake was assessed by asking patients about diet and any vitamin D supplementation and estimates of total solar irradiance was calculated based on the geographic location of the clinic centers and controlled when interpreting the results. At each clinic visit, depressive symptoms were assessed using the Burnam 8-item scale for depressive disorders. When controlling for confounding factors, they found that lower vitamin D intake of <100 IU/day had a strong inverse correlation in women who met criteria for prevailing depressive symptoms at the baseline visit. And the reverse was also true, those patients with a substantial intake of vitamin D was associated with a lower prevalence of depressive symptoms. This effect seemed to have a threshold, as those positive effects of increased intake was only shown in patients who consumed 400 to <800 IU/day and an intake of ≥800 IU/day was not associated with decreased risk of depression. At year 3 of the study increased vitamin D intake was correlated with lower risk of depressive symptoms in patients with no evidence of depression at baseline. A limitation of this study, is that it did not measure serum 25-hydroxyvitamin D levels in conjunction with intake levels. This is important as the effects of vitamin D intake on serum vitamin D levels may differ in individuals. Individuals may differ in the processing of vitamin D ingestion; these differences are in part because of genetic factors, vitamin D metabolism and general dietary intake. This study saw an inverse relationship between vitamin D intake and depressive symptoms in older women.

One study, looking at the direct effects of vitamin D supplementation on depressive symptoms in conjunction with metabolic profiles, c-reactive protein (CRP), an inflammatory marker, and oxidative stress. Sepehrmanesh et al., (2016) performed a randomized, double-blind, clinical trial in performed in Kashan, Iran, from October 2014 to December 2014. They included 36 patients, ages 18–65 years, equal parts men and women, diagnosed with major depressive disorder and randomly assigned 18 of them to receive a single capsule of 50,000 IU vitamin D weekly and 18 patients to receive a placebo weekly for 8 weeks in total. The Beck Depression Inventory (BDI) was used to assess depressive symptoms before and after supplementation. As expected, after 8 weeks, changes in vitamin D concentrations were greater in the vitamin D group than in the placebo group. The group taking vitamin D supplements had improved insulin function and decreased oxidative stress than the patients that took a placebo. No changes were seen in c-reactive protein levels between both groups. This may be because of the duration of the study or the specific inflammatory marker studied, as different inflammatory markers have been found to be associated with vitamin D deficiency and depression. But most importantly, this study found that the patients taking vitamin D supplements had significant decreased depressive symptoms, and decreased BDI total scores, post-nutritional therapy compared to before. As all these patients had vitamin D deficiency of less than 20 μg/L at the start of this study, it is not possible to assess if the effects of supplementation are seen only in vitamin D deficient individuals or this therapy may also be useful in those who have clinically sufficient levels of vitamin D. Perhaps the beneficial effects of supplementation can be explained by the fact that both groups had a low baseline mean serum 25-hydroxyvitamin D level. Therefore, a relatively short supplementation period was effective in lowering depressive symptoms. Overall, vitamin D supplementation had a positive effect on lowering depressive symptoms in patients with major depressive disorder.

Conclusion

Vitamin D status seems to be directly linked to mood disorders and particularly major depression. A mechanism for this association is demonstrated in detail, as both are correlated to inflammatory cytokine status. Vitamin D deficiency is associated with an elevation of proinflammatory cytokines because vitamin D modulates and prevents uncontrolled inflammation systemically and in the central nervous system. This elevation of proinflammatory cytokines may have adverse psychiatric effects. It can contribute to depressive symptoms and even suicide. Patients with suicidal thoughts specifically, seem to have abnormal proinflammatory cytokines and lower levels of vitamin D in their body, than patients with major depression that do not have suicidal thoughts.

As the conceptual understanding of vitamin D's activity as a neuro and immune modulator on the brain is an innovative and relatively new area of study, there are few direct-link studies on how the supplementation of vitamin D may reduce instances of major depressive disorder and suicide risk. Vitamin D supplementation treatment for major depressive disorder should be tested in a larger sample size, in patients with clinically sufficient vitamin D status, and for longer periods of time. Additionally, this treatment should be tested in patients with suicidal ideation, as this is the subgroup that has been differentiated to be effected most by vitamin D deficiency. Furthermore, studies should assess the difference between treatments that raise vitamin D levels by intake versus UV ray exposure. Perhaps, vitamin D status and its effects may differ when synthesized naturally in the skin with direct access to subcutaneous fat for storage and the bloodstream, in contrast the supplementation being first processed in the gastrointestinal tract. Vitamin D as a treatment method for major depressive disorder is a new and exciting area of research that may impact the global population tremendously due to the large number of people suffering of depression worldwide. More research on the effectiveness and limitations of this treatment should be done to maximize its impact.

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Do Antbiotics in Early Life Contribute to Obesity?

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Abstract

In recent years, science has made great strides in its understanding of the microbiome, discovering that it plays a role in regulating many body processes. One area of study is the microbiomes interaction and influence on host metabolic processes. Studies using both mice and humans have established a clear correlation between obesity and the composition of the microbiome, identifying a microbiome unique to obese individuals. Furthermore, experiments with germ-free mice have shown that the microbiome effects host metabolism, causing germ free mice to increase in mass when inoculated with normal microbiota. Inoculation with microbiota from obese mice yielded greater increases in mass, showing the obesogenic effect of the microbiota. The mechanisms through which the microbiome can contribute to obesity are enhanced extraction of energy from food, and increased capacity for nutrient uptake in the gut, and alteration of metabolic pathways by suppressing fasting induced adipose factor and decreasing AMPK activity. Many of these pathways show increased activity in obese mice. The enhanced energy extraction coupled with greater deposition of fat mediated by altered metabolic pathways can contribute to obesity.

The role of the microbiota in obesity, combined with decades-old observations that antibiotics, particularly early in life, increased the weight of livestock, led to a hypothesis that antibiotics can disrupt the development of the microbiome, causing metabolic changes, leading to obesity. Recently, this hypothesis has been tested, both in studies utilizing mice, and in many epidemiological studies. This paper will evaluate the available evidence to determine if exposure to antibiotics early in life can lead to increased incidence of obesity later.

Introduction

In 1671 when Antony van Leeuwenhoek first spied "animalcules," now known to us as single cell organisms, through his homemade microscope, he set into motion a centuries long scientific endeavor to discover, describe, catalogue and gain a deeper understanding of the vast microbial world that surrounds us. This laid the groundwork for the development of the germ theory by Louis Pasteur and Robert Koch over the course of the 1860's and 1870's. Germ Theory, the theory that many diseases are caused by microbial agents, revolutionized medicine leading to many advances such as the disinfecting of wounds, the appreciation of the need for a sterile environment during surgery, and in 1921 the fortuitous discovery of penicillin by Alexander Fleming. Germ theory also had a profound influence on the attitudes of medicine and society at large towards "germs." Although only a tiny fraction of microbes are pathogenic, they became indelibly associated, both in the popular imagination and in medical practice, with sickness and disease. This focus on microbe pathogenicity has yielded tremendous benefits to public health, in fact antibiotics were among the primary drivers of rising life expectancy in developed countries in the mid twentieth century (Armstrong, 1999), but antibiotics, for all their benefits, came with costs as well, namely their effect on the native flora.

Discovery of the body's native flora began in the mid 1880's when an Austrian pediatrician, Theodore Escherich, observed the eponymously named Escherichia coli in the stool of healthy children. Discovery continued apace and the realization set in that there was a large, diverse, community of microbes that colonized the skin, nasal and oral cavities, and the urogenital and gastrointestinal tracts of healthy people, making up their native flora. As early as the 1970's there was already an idea of the number of microbes, then estimated to number roughly 10^{14}, living primarily in the gastrointestinal tract, and some idea of ecology of this diverse community (Savage, 1977). Until relatively recently, it was assumed that the microbes living in the colon lived a largely commensal existence, dining on food indigestible to the human host but not interacting with the host in any meaningful way. With this understanding collateral damage to the gut bacteria because of antibiotics was no great concern, with the worst-case scenario being an unwanted bloom of C. Difficile. However, in recent years and particularly since the launch of the Human Microbiome Project, an entirely different picture has emerged, one that includes many symbiotic relationships between the native flora and the host, in fact so enmeshed is the host-symbiont relationship, that they have been described as one "supraorganism (Turnbaugh, et al., 2007). This new understanding demands a closer look at the possible effects of antibiotics on our microbiome.

This paper will explore the possible link between antibiotics and obesity. First it will lay the groundwork with a thorough exploration of the literature linking the state of the microbiome to obesity as well as an understanding of the underlying mechanisms. Then it will consider the evidence that a disturbance of the microbiota through antibiotic treatment can cause obesity along with proposed causative mechanism. Finally, it will propose ways to mitigate the effects of the antibiotic treatment.

Methods

Information for this paper was obtained primarily through online searches utilizing google scholar as well as well as numerous databases accessed through Touro college's library system.

Definition of "Core Microbiome"

The first step to determining if obesity is associated with an altered microbiome is establishing the baseline values defining what a "normal" gut microbiome looks like. This task is complicated by the wide diversity of gut microbial populations found across different geographical areas and cultures, and even within communities and cultures. In fact, in one study "there was not a single abundant (defined as >0.5% of the community) bacterial species shared by all 154 individuals" involved in the study (Turnbaugh P. J., 2009) . However, if one looks at the microbiome on the level of phyla, a strong pattern begins to emerge, with bacteria from the phyla Firmicutes and Bacteriodetes representing, in one study, 92.6% of the microbiota (Ley, et al., 2006). These 2 phyla and their respective ratios can serve as one definition of a core microbiota.

Another way of defining the core microbiome is, rather than focusing on the species or phyla present, focusing on the various genes present. Various studies utilizing this methodology have found that regardless of the vast diversity of the microbial makeup of the gut flora, there exists a "wide array of shared microbial genes, comprising an extensive, identifiable 'core microbiome' at the gene, rather than at the organismal lineage, level" (Turnbough, et al., 2009). While both definitions are useful, this paper will primarily utilize the second definition of the microbiome as a set of genes rather than ratio of different bacterial phyla. The reason for this is that a focus on genes can better illuminate any products of the microbiome that may affect host metabolic pathways, possibly contributing to obesity.

Association of Obesity with Altered Microbiome

Having established a baseline microbiome, we can now explore any obesity associated changes that may occur. First, we will explore the changes in the ratio of Firmicutes and Bacteroidetes associated with obesity.

In one experiment, mice heterozygous for obesity ob/+ (due to a defective leptin gene) were mated producing litters consisting of a mix of obese (ob/ob) and lean (ob/+ and +/+) phenotypes. Microbial ecology in the gut, specifically the ratio of Firmicutes to Bacteroidetes, which is typically similar among members of a family living together, was found to be consistent in the heterozygous mothers as well as the ob/+ and +/+ children. In the homozygous ob/ob mice of the same litter however, a sharp increase in abundance of Firmicutes relative to Bacteroidetes was observed (Ley, et al., 2005). This shows a clear correlation between obesity and the composition of the gut microbiome.

In another experiment, 12 obese individuals were randomly placed on either a fat or carbohydrate restricted diet and their gut microbiota was monitored over the course of a year for any discernible shift in the microbiota as they lost weight. Initially, obese people had more firmicutes and fewer Bateroidetes than lean controls. However, over the course of the year as their weight dropped, the ratio of Firmicutes to Bacetoidetes began to more closely resemble a typical lean profile (Ley, et al., 2006).

Together, these two studies, encompassing both mice and men and showing both an increase in abundance of Firmicutes as an obese phenotype was acquired in the mouse experiment and a decrease in its abundance as weight was lost in the human experiment, firmly establish that obesity is associated with an altered microbiome.

Can the Gut Microbiome Cause Obesity?

Having established a strong correlation between obesity and altered gut microbial ecology, we can now explore the possibility that the microbiome can be a causative agent in obesity.

There seems to be good experimental evidence, at least with mice, that this is the case.

In one experiment, mice were divided into three groups. One group was raised "Germ Free" meaning that their gut was sterile. Another group consisted of regular, conventionally raised mice, and acted as a control. A third group was initially raised germ, free but subsequently inoculated with gut bacteria at 7-10 weeks by spreading a suspension of cecal content from the control group mice on their fur. Comparison of germ free and regular mice at 8 to 10 weeks found that regular mice had 42% more body fat than their germ-free companions and had epidydimal fat pads weighing 47% more, all while eating 29% less food. After a 14-day colonization, a process known as conventionalization, the third group of mice experienced a dramatic 57% increase in total body fat and a 61% increase in epididymal fat pad weight all while their chow consumption decreased to that of the normal mice (Backed, et al., 2004). This experiment shows that gut microbiota has a powerful effect on metabolism and fat storage. The initial low-fat state of the germ-free mice even with their above average food intake and their dramatic increase in fat, even in the face of decreased chow consumption, as they were conventionalized, indicates that a normal microbiome plays a key role in regulating fat in mice.

Having established the effects of a normal microbiome, let us examine the effects of an obese one. Toward this end, an experiment was constructed in which germ-free mice were colonized by gavage (meaning they were fed by tube) with the cecal contents of either wild type +/+ or genetically obese ob/ob (leptin deficient) mice. In the 14-day period following the colonization food consumption in the (ob/ob) and (+/+) transplanted groups was not statistically different (55.4±2.5g for ob/ob against 54.0±1.2g for

+/+) and they both ate the same type of chow (no difference in caloric density). Despite this, the mice colonized with ob/ob microbiota exhibited a significantly greater increase in body fat than those colonized with +/+ microbiota with the ob/ob colonized mice increasing body fat by 47±8.3% and the +/+ colonized mice increasing by just 27±3.6% (Turnbaugh,et al., 2006).

The dramatic difference in body fat between the two groups strongly indicates that the obese microbiome causes greater adiposity, and gives rise to the possibility that the microbiome can play a role in its development.

Mechanisms of Microbiome Influence on Adiposity

The classic, somewhat simplistic understanding of the development of obesity is to take the calories of the food eaten, subtract calories burned by both the basal metabolic rate and any additional energy expenditures for various activities, and assume that the remainder is stored as fat in adipose tissue throughout the body. Our exploration of the mechanisms through which the gut microbiota increase adiposity will illuminate several ways that this seemingly straightforward and commonsense equation can be altered.

One mechanism proposed is that all microbiomes increase the bodies capacity for energy harvest from food eaten by excreting exoenzymes that break down polysaccharides that the host is unable to metabolize. Once degraded into monosaccharides and short chain fatty acids, both the bacteria and the host readily take up the product, accruing extra calories to the host from the same food. It is further hypothesized that the changed obese microbiome performs these tasks more efficiently extracting even more calories from the same unit of food with the host reaping some of the benefits. This hypothesis is buttressed by numerous lines of evidence.

The first of these is a simple comparison of the energy remaining in the feces of regular mice as opposed to genetically obese mice. Bomb calorimetry showed that ob/ob mice have significantly less energy remaining than regular mice, yielding 3.2 kcal/g compared to 3.4 in regular mice (Turnbaugh, et al., 2006). This is simple, clear, empirical evidence that an obese microbiome harvests more energy than a standard, lean microbiome.

Another line of evidence involves a genetic analysis of the microbiome, specifically of genes encoding enzymes that catalyze the breakdown of polysaccharides indigestible to their hosts. In one study, a sequencing of 18 Human microbiomes identified genes for 156 carbohydrate- active enzymes, which are enzyme families that break down carbohydrates, including 77 glycoside hydrolase, 21 carbohydrate-binding module, 35 glycosyltransferase, 12 polysaccharide lyase and 11 carbohydrate-esterase families. These genes consisted of fully 2.62±.013% of all the microbial genes sequenced, a higher percentage than any other identified group of genes. Furthermore, an analysis of lean and obese twins found that the obese twins had a microbiome that was significantly enriched in genes coding for carbohydrate, lipid, and amino acid metabolism as compared to that of their lean twins (Turnbough, et al., 2009).

Mice studies have yielded similar results, with ob/ob mice having microbiomes containing more genes coding for various carbohydrate-active enzymes as compared with their lean littermates. A predicted result of this would be an increased concentration of the products of bacterial fermentation of these polysaccharides, such as butyrate and acetate, in the cecum of the ob/ob mice. This prediction was borne out, with cecal butyrate concentration of obese mice double those of lean mice and acetate levels 20% higher (Turnbaugh, et al., 2006).

Interestingly this same study also found a greater abundance of archaea in the obese mice than their lean counterparts. Archaea oxidize the hydrogen produced as a by-product of fermentation by gut bacteria, turning it into methane. By removing a product of the fermentation reaction, they increase its efficiency, serving to further enhance energy extraction by the obese microbiome. Indeed, in a study of mice colonized with archaea commonly found in the human gut, Methanobrevibactor smithii and B. thetaiotamicron, a significant increase in the efficiency of bacterial polysaccharide fermentation leading to an increase in adiposity in the mice was observed (Samuel & Gordon, 2006)

In addition to increasing energy extraction from food, there is also evidence that the microbiome increases the hosts capacity for uptake of nutrients in the gut. In one experiment, germ-free and conventionalized mice were fed a glucose solution. After fifteen minutes, the level of glucose uptake was found to be twice as high in the conventionalized mice as in the germ-free ones (Backed, et al., 2004). Additionally, the microbiome is essential to the development of the capillary network to transfer these nutrients from the intestines to the hepatic portal vein. Germ-free mice were found to have arrested development of this capillary network, and upon conventionalization, developed it to normal levels within ten days (Stappenbeck & Hooper, 2002).

These lines of evidence collectively paint a picture of a microbiome that extracts more energy from food by breaking down complex polysaccharides that the host is unable to metabolize on his own and amplifying the hosts ability to absorb the resultant monosaccharides, providing one possible mechanism for the microbiome to contribute to obesity.

Another mechanism proposed is that the microbiome modifies cell signaling pathways to increase fat storage, that is, to direct more of the energy harvested toward adipocyte storage rather than other metabolic functions. Two metabolic pathways are involved, one of which involves fasting-induced adipose factor (Fiaf) which is a lipoprotein lipase inhibitor (Backed, et al., 2004). Lipoprotein lipase facilitates deposition of fat in adipocytes. Fiaf, which inhibits it, is a crucial regulator of this process. Fiaf is produced by brown and white fat, the liver, and the intestine. The microbiome suppresses the production of Fiaf in the intestinal epithelium, thereby increasing the activity of lipoprotein lipases, resulting in more triglycerides being incorporated into adipocytes.

Experimental evidence for this machanism comes from a study that compared regular germ-free mice, germ-free mice incapable of producing Fiaf (Fiaf-/-), and conventionalized mice both with and without the Fiaf gene. The regular germ free mice, as expected were the leanest. Germ free Fiaf-/- mice however, were found to have nearly the same amount of fat as their conventionalised wild type peers. Furthermore, a conventionalization of the germ free Fiaf knockout mice yielded a minimal increase in body fat of 10±8% versus 55±16% for the wild type germ free mice. Conventionalization of heterozygotes (Fiaf +/-) yielded an intermediate result, consistant with the hypothesis. Additionally comparison of mRNA of conventionalised and germ free wild type mice revealed camparitively less Fiaf expression in the small intestines of the the former, expression elswhere though was unaffected (Backed, et al., 2004). Other studies have had similar findings, including one that found that while regular germ free mice were resistant to obesity induced by consuming an "american diet" in their case chow with higher fat content and more easily digested sugars, Fiaf -/- mice had lost this resistance (Backhed, et al., 2007). These findings point to fasting induced adipose factor as a major component of the microbiomes contribution to adiposity.

Another pathway involves levels of AMP-activated protein kinase, or AMPK (Backhed, et al,. 2007). AMPK is a key enzyme regulating metabolism, serving as the lynchpin of a complex web of metabolic pathways maintaining proper ATP levels. AMPK ramps up energy production in response to metabolic stresses. It is triggered primarily, as its name indicates, by an increased ratio of AMP to ATP, but also by numerous other factors such as an elevated ratio of NAD to NADH, and the hormones leptin and adiponectin (Kahn, et al,. 2005) . The microbiome is thought to decrease AMPK activity, leading to lower energy expenditure, with more calories remaining to be deposited as fat.

Evidence for this mechanism is based on a number of observations. The first is that germ free mice were found, using an immunoblot assay, to have phospho-AMPK, which is the active form, at concentrations 40% percent greater than their regular peers in their gastrocnemeus muscles. Consistant with these findings AMP levels in the germ-free mice were foud to be 50% higher. Additionally, many other enzymes involved in the fatty acid oxidation pathway triggered by AMPK showed fluctuations consistant with increased fatty acid oxidation. In this pathway, AcetylCoA carboxylase converts Acetyl CoA to Malonyl CoA, Malonyl CoA inhibits carnitine-palmitoyl transferase-1 (Cpt1), which catalyzes the rate-limiting step for uptake of long chain fatty acids by mitochondria, AMPK phosphorylates AcetylCoA carboxylase, inhibiting it and thereby increasing fatty acid oxidation (Kahn, et al, 2005). A 43% increase in the levels of phosphorylated AcetylCoA carboxylase was found using an immunoblot assay and a 17% increase in the level of Cpt1 was found with a biochemichal assay, in germ free over that of regular mice,both consistant with increased fatty acid oxidation (Backhed, et al,. 2007).

Collectively, these lines of evidence paint a picture of a microbiome that acts on both sides of the energy equation, harvesting more energy from food through greater polysaccharidase activity, conserving more of that energy through, and depositing a greater portion of it as fat.

Can Antibiotics Contribute to Obesity?

Having gained some appreciation of the influence of our microbiota on our metabolism and its role in promoting adiposity, we can now explore the role of antibiotics on this complex system. Specifically, we will explore whether the disruption to the microbiota caused by antibiotics, particularly early in life, can affect the body mass of the host later in life by either promoting or inhibiting weight gain.

There is extensive evidence that antibiotics promote weight gain, from veterinary medicine, animal models, and epidemiological studies.

In the 1950's Veterinary scientists showed that giving pigs (Taylor & Gordon, 1955) and other livestock (Jukes & Williams, 1953) sub-therapeutic doses of antibiotics increased their growth causing them to gain more weight without increasing feed consumption. It subsequently became common practice among farmers to mix low doses of antibiotics into the feed of pigs, cows, sheep, and poultry, increasing their weight, a practice that continues to this day. The effect on weight gain is significant, a meta-analysis of numerous studies gauging the weight boosting effects of adding antibiotics to feed in pigs found an increase in weight gain of up to 15% and an increase in feed efficiency (an industry term for amount of meat produced per unit of feed) of up to 6%. The strongest effects were found when the antibiotics were given from birth with lesser, though still significant effects

found if they were given at later dates (Hays, 1969). Evidence that the weight gain is connected to disruption of the microbiota comes from experiments with germ free chickens. Germ free chickens given feed containing antibiotics that had a growth promoting effect on regular chickens showed no similar increase in growth from the treatment (Coates, 1963), indicating that the weight gain is a result of modulation of the microbiome. These veterinary studies on regular and germ free animals, combined with the everyday experience of farmers for the last 70 years provide one line of evidence that antibiotic use has a role in weight gain. Importantly, as the data showed, the effect is greatly magnified in early life, suggesting that the early microbiome may be particularly vulnerable to whatever disruption causes the weight gain, a theme that will be expanded on shortly.

More evidence comes from experiments with model organisms, namely Mus Musculus, the mouse. Additionally, these experiments shed light on the magnified effects of antibiotics found in early life.

In one experiment the effects of sub-therapeutic antibiotic treatment (STAT) was tested on mice to attempt to replicate its observed effect on farm animals and gain some insight into its mechanisms. In the experiment mice were exposed, starting at weaning, to various common antibiotics by putting them in their drinking water at sub-therapeutic levels, and were compared to a control group using various metrics. After a seven-week exposure, the STAT mice were found to have greater fat mass than the control group as well as a significantly higher percent body fat. Curiously although fat mass was greater in the STAT mice, total mass was not significantly greater at seven weeks, though later measurements taken from 8 to 26 weeks did show increased mass in the STAT mice (Cho, et al., 2012).

Utilization of other metrics allows for a deeper understanding of the processes driving the adiposity of the STAT mice. One important measure taken was the level of Gastric Inhibitory Polypeptide (GIP). GIP, a hormone secreted by K cells in the small intestine, stimulates lipoprotein lipase activity, increasing fat storage and contributing to adiposity (Miyawaki, et al., 2002). GIP levels were found to be substantially elevated in STAT mice (39.1±2.5 pg/ml) compared to the controls (24.4±4.2 pg/ml). This provides a possible mechanism for the observed increase in adiposity.

Microarray analysis of differential gene expression in hepatic tissue yielded deeper insights into the metabolic changes wrought by STAT. Comparison of STAT and control mice found upregulation of pathways for lipogenesis and triglyceride synthesis in the STAT mice, further contributing to adiposity.

Examination of the gut bacteria in the STAT mice yielded further insight. Although the overall number of bacteria did not change significantly, the composition of the microbiome did change, with the abundance of Firmicutes increasing relative to that of Bacteroidetes, which, as discussed earlier, is typical of obese microbiomes. Additionally, examination of the cecal contents of the STAT mice found higher levels of butyrate and acetate, suggesting increased energy capture through fermentation of complex carbohydrates indigestible to the mice, as discussed earlier. Supporting evidence came from metabolic cage experiments showing no difference in caloric intake but a lower caloric output in fecal pellets in STAT mice compared to controls (Cho, et al., 2012).

Taken together, these measurements paint a picture of antibiotics changing the composition of the microbiome, leading to metabolic changes causing adiposity and weight gain, and suggest that perhaps antibiotics can contribute to obesity in humans as well.

Increased Effect in Early Life

Greater weight gain was observed in farm animals when STAT was started earlier in life. Mouse studies have explored the importance of the timing of antibiotic exposure further, experimentally confirming these observations and expanding upon them. They found that early life is a critical time in metabolic development and exposure to antibiotics at this sensitive stage can permanently alter host metabolism.

Evidence for these claims comes from an experiment comparing mice started on low dose penicillin (LDP) at weaning (LDP-w) to mice where LPD was started shortly before birth(LDP-b) so that the initial colonization with maternal microbiota would be altered. A control group was maintained that was not exposed at all.

The experiment found that earlier administration of antibiotics did have amplified effects. The growth rate for LDP-b was greater than the control, the fat mass as well as the total mass of adult LDP-b male mice was greater than that of LDP-w mice and the control (Cox, et al., 2014), demonstrating enhanced effects of earlier antibiotic administration. Sexual dimorphism was apparent in the results with the females experiencing lesser if any effects, a finding that remains unexplained. Metabolic differences between the LDP-b and LDP-w mice were found as well with the LDP-b mice having greater expression of genes involved in adipogenesis than LDP-w mice.

The mammalian early microbiome is a dynamic, changing environment typically showing a pattern of succession as different taxa first dominate then diminish (Pantoja-Feliciano, et al., 2013). Altered representation of some of these taxa has been

associated with obesity (Kalliomaki, et al,. 2008). Typically, Lactobacillus is prominent in nursing animals, as was indeed the case with the controls. The LDP-b mice however, showed much lower levels of Lactobacillus, as well as other groups whose population typically peak in early life such as Candidatus Arthomitus and Allobaculum (Cox, et al., 2014). Although the precise roles of these microbes are not known, their suppression by LDP and the dramatic phenotypic effects that follow suggest some role in metabolic development.

In an experiment with worrying implications for human obesity, some LDP mice were switched to high fat diet at 17 weeks, and were compared to control groups with just a high fat diet or just LDP. The growth promoting effects of LDP were accentuated by the high fat diet producing fat and weight gain surpassing that produced by the high fat diet or LDP alone.

More worrying still, the metabolic effects of LDP lasted into adulthood even after treatment finished. Mice that received LDP for only four weeks after birth still experienced greater fat and total mass accumulation from 6-20 weeks. This weight gain persisted even though the microbiota had appeared to normalize.

Finally, to demonstrate that the metabolic and phenotypic changes observed were a result of an altered microbiota and not some direct effect of the penicillin, cecal microbiota were transferred from 18-week-old control and LDP mice to 3 week germ-free mice. The mice inoculated with the LDP microbiota increased total mass and fat mass at a faster rate (.078 g/day total mass and .058 g/day fat mass faster) than those inoculated with the normal microbiota (Cox, et al., 2014).

These studies provide convincing evidence, as much as can be inferred from model organisms, that antibiotics contribute to obesity through disruption of the microbiota, and that early life is a particularly sensitive time when disruption of the developing microbiome can have long lasting metabolic effects.

Epidemiological Studies

The findings in model organisms that antibiotic exposure, particularly in early life, can lead to obesity, have important implications for human health. However, results in model organisms do not always translate into results in humans. Since ethical concerns preclude the types of randomized, controlled studies routinely performed with model organisms from being done on humans, we must rely on epidemiological evidence. Fortunately, there are a wealth of well-constructed epidemiological studies demonstrating that antibiotic exposure in infancy is correlated with obesity later in life.

In a study involving 28354 mother baby pairs from the Danish National Birth Cohort, antibiotic exposure in the first six months of life was correlated (with an odds ratio of 1.54, well above the threshold for showing correlation) with an increased risk of being overweight at 7 years (Ajslev, et al., 2011). Supporting these findings are results from a study utilizing 11532 children from the Avon Longitudinal Study of Parent and Children. This study examined antibiotic exposure during three early-life time windows, 6< months, 6-14 months, and 15-23 months. Exposure under six months was, once again, strongly correlated ($p<.001$) with increased body mass at 10, 20, and 38 months. However, exposure from 6-14 months showed no effect and exposure at 15-24 showed a modest weight gain at 7 years (Trasande, et al., 2013). A Canadian study combining data from health records and a Canadian longitudinal birth cohort study further bolstered these findings. The study found that children who received antibiotic treatment in the first year of life were more likely to be overweight at ages 9 and 12 than their untreated peers (32.4% overweight if exposed vs. 18.2% if not). Additionally, researchers noted a greater prevalence of elevated central adiposity, a precursor of metabolic syndrome, among the treated children (Azad, et al, 2014). A longitudinal study in the USA (Bailey, et al., 2014)and a global cross sectional study (Murphy, et al., 2013) found similar results as well. Several the studies (Trasande, et al., 2013) (Murphy, et al., 2013) found a strong sexual dimorphism, with the effect much greater in boys, and nearly all the studies showed some difference between girls and boys, with the boys seeing greater weight gain than the girls, a finding that while replicated many times in both model organisms and in humans, has not been satisfactorily explained.

The collective weight of these epidemiological studies gives great credence to claims that antibiotics contribute to obesity.

Mitigating the Effects of Antibiotics

Even with all the evidence of detrimental side effect of antibiotics, stopping their use is obviously not an option. Antibiotics are a cornerstone of modern medicine, without which life expectancy would surely drop precipitously. However perhaps a bit of restraint in prescribing antibiotics to children is in order. Rates of antibiotic prescriptions in the USA are unnecessarily high, with some analyses finding that fully half of all antibiotic prescriptions written are unnecessary (Nyquist, et al., 1998). Although antibiotic prescription rates among children and adolescents have dropped since that finding (Lee, et al., 2014), prescription rates in the USA are still high compared to some other countries. In Sweden, for example, antibiotic use is 53% lower than in the USA (Ternhag & Hellman, 2010). This indicates that prescription levels can still be lowered significantly without adversely effecting public health. While some antibiotic exposure may be unavoidable for many children, even merely reducing the

number of rounds of antibiotics they take reduces their chance of developing obesity later in life (Bailey, et al., 2014).

One final action possible to mitigate the obesogenic effects of antibiotics on children is to prescribe narrow spectrum antibiotics when possible. One study, despite finding significant correlation between broad-spectrum antibiotics in the first two years of life, found no such correlation for narrow-spectrum antibiotics (Bailey, et al., 2014).

Conclusions

In conclusion, the evidence for an obesogenic effect of early-life exposure to antibiotics is substantial and convincing. The many mouse studies demonstrating the influence of the microbiome on metabolism and its role in obesity give ample reason to suspect that perturbations of the microbiome with antibiotics may have some effect on obesity. The evidence from farm animals, mice, and epidemiological studies serve to confirm that suspicion, showing that antibiotic exposure in infancy contributes to one's chances of developing obesity later in life.

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